INVESTIGATION OF A SELF-MODULATED FLUORIDE RELEASING ADHESIVE FOR DENTAL APPLICATIONS

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1998

To my parents and brother.

ACKNOWLEDGMENTS

This work was supported by NIH/HIDR Grant P50 DE09307.

I would like to express my deepest gratitude to my advisor and mentor, Dr. Christopher D. Batich, for his thoughtful guidance, assistance, and most importantly, his encouragement and support whenever I faced difficulties during these years of stay. I can not dream to have a better boss than this. Special thanks to my other committee members, Dr. Eugene P. Goldberg, Dr. Anthony B. Brennan, Dr. Chiayi Shen, and Dr. Kenneth B. Wagener, for their valuable advice and excellent critiques. I would like to thank the following colleagues: Travis Arola for his help with fluoride release study; Mr. Eduardo Mondragon for his help with preparation of samples for shear bonding strength test; Dr. James Marotta for his assistance with XPS analysis; and Dr. Nicola Richards for giving me informative knowledge in dentistry, and assisting me in many occasions during these years of stay.

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

INVESTIGATION OF A SELF-MODULATED FLUORIDE RELEASING ADHESIVE FOR DENTAL APPLICATIONS

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December, 1998

Chairman: Christopher D. Batich
Major Department: Materials Science and Engineering

Recurrent caries, a secondary carious attack after the treatment of primary caries, remains one of the primary causes for the failure of dental restorations. Due to the current limitations of detecting recurrent caries, a large proportion of dental fillings are replaced unnecessarily. During the carious attack, the local pH decreases due to the metabolism of cariogenic microflora. Fluoride plays an irreplaceable role in the prevention of dental caries by remineralization and suppression of growth of cariogenic flora. This work investigates the possibility of using a dental adhesive incorporated with fluoride-loaded pH-sensitive microspheres for localized self-modulated fluoride release.

A novel ion-exchange-based fluoride loading technique was developed. A detailed study of fluoride loading level as a function of composition of loading medium, fluoride concentration in loading medium, and duration of ion exchange, was presented.

Several methods were used to determine fluoride loading in this study. The limitations of these methods were compared and discussed.

pH-sensitive fluorine release from a fluoride-loaded pH-sensitive copolymer was investigated in 0.1 M lactic buffers. The influence of fluoride loading level on the pH sensitivity of the copolymer was investigated. A pH-sensitive fluorine release was observed from the copolymer with a low fluoride loading level. Release onset pH is close to the critical pH for dissolution of dentin (pH 5.5). pH sensitivity of the copolymer diminishes at a high fluoride loading level.

A series of BisGMA/HEMA-based adhesive resin mixture was prepared. Shear bond strength of the resin mixture on human dentine was tested. The influences of BisGMA-to-HEMA on the shear bond strength and the fluorine permeability have been studied. The highest shear bond strength (28.8 ± 5.2 MPa) was observed for the resin with 50 %wt BisGMA. The fluorine release rate decreases markedly with increasing BisGMA content in the resin.

Finally, the influence of presence of the microspheres in BisGMA/HEMA resin mixtures on the shear bond strength was also evaluated. Although a decrease in shear bond strength was observed, this adhesive system could still be valuable for the delivery of fluorine ions to prevent the white spot lesions from appearing around orthodontic brackets, where the bond strength is less demanding.

CHAPTER 1
INTRODUCTION

In the industrialized countries dental caries is one of the most ubiquitous and costly illnesses. The total cost of treating oral diseases exceeds the cost of treating any other single disease entity (Nikiforuk, 1985). Further, treatment alone does not confer immunity from subsequent attack. It is becoming increasingly clear that the best hope for reducing the burden of dental illnesses is through prevention.

Fluoride plays an irreplaceable role in the prevention of dental caries. There is no doubt of the anti-caries effectiveness of fluoride, taken either systemically during the period of tooth formation and mineralization, or applied topically after eruption of the teeth. There are extensive data to show that the prevalence of dental caries has declined in most industrialized countries in the past two decades. A recent report of the World Health Organization concluded that this decline can be attributed mainly to the widespread use of fluoridated dentifrices (WHO, 1994). Unfortunately, recurrent caries remains one of the primary causes for replacement of dental restorations (Mjör, 1981).

Recurrent caries is a secondary carious attack after the treatment of primary caries. It commonly occurs in dentin beneath or around the restoration. Because of the current limitations of dental restorative materials and techniques, a restoration is often associated with leakage along the tooth-restoration interfacial region. This mismatch in the physical properties between dentin and synthetic materials generally leads to a

microleakage gap which may slowly undermine existing restorations and lead to recurrent caries. Not only is a high incident rate associated with the recurrent caries, but the diagnosis of the recurrent caries is also severely limited. To date, clinicians can only rely on the explorer, mirror and radiograph to detect recurrent caries (Kidd, 1990), and the detection rate is about 64%, slightly better than a random guess (Tveit et al., 1991). Clearly, improved methods of preventing and diagnosing recurrent caries are needed.

Research shows *Streptococcus mutans*, along with other bacteria, is one of the primary factors in the caries etiology. With a suitable substrate, these bacteria produce acids (mainly lactic acid) as byproducts of metabolism, which directly causes the demineralization of the dentin and enamel. The demineralization of the tooth structure could further lead to the dental caries. As generally accepted, the demineralization of the dentin starts at pH 5.0 - 5.5 (Larsen and Bruun, 1994).

A local decrease in pH indicates a potential carious attack. It may thus serve as a biological signal calling for release of an anticarious drug, such as fluoride, from a pH-sensitive controlled drug release device. We have successfully developed different types of pH-sensitive microspheres based on vinylpyridine, N, N-dimethylaminoethyl methacrylate (DMA); N,N-diethylaminoethyl methacrylate (DEA); methyl methacrylate (MMA); styrene (St) and divinylbenzene (DVB) (Batich et al., 1993; Wei, 1995; Yan and Batich, unpublished). The molecular structure of these monomers is shown in Appendix A. Based on St/DMA/DVB microspheres, we demonstrated a pH-sensitive swelling of the copolymer in four different buffer systems. The pH-sensitive release of 9-aminoacridine (a dye) was closely related with the swelling behavior of the copolymer matrix (Wei, 1995). The study showed that the release onset from the microspheres

containing 30%mol DMA was varied in different buffers within the range of pH 4.5-6.0. The study described within this dissertation was designed to apply pH-sensitive controlled release technology to self-modulated release of fluoride in carious dentin. Generally, recurrent caries is not likely to progress immediately after the initial restorative treatment. Therefore, a self-modulated release therapeutic agent is crucial for the prevention of recurrent caries. The goal was to develop a novel dental liner material which would release fluoride once the local environment became acidic, indicating the presence of cariogenic bacterial attack. The ideal release onset pH is around 5.5. That means, this dental liner material should retain the loaded fluoride at a pH above 6.0, and release it at a pH of 5.5 or less in a controlled rate. Figure 1.1 illustrates a graphic representation of the ideal release characteristic of fluoride from the liner materials. Since the liner material would be applied between the dentin and the restorative material, the bond strength at the interface of dentin and restorative material is also crucial to ensure the longevity of the restoration.

The more specific goals of this work were to

1) develop methods of loading the fluoride into the lightly cross-linked pH-sensitive copolymer microspheres.
2) develop a light curable bonding agent which also served as diffusion barrier to regulate the fluoride release rate.
3) investigate the bond strength of the fluoride release liner material on human dentin.

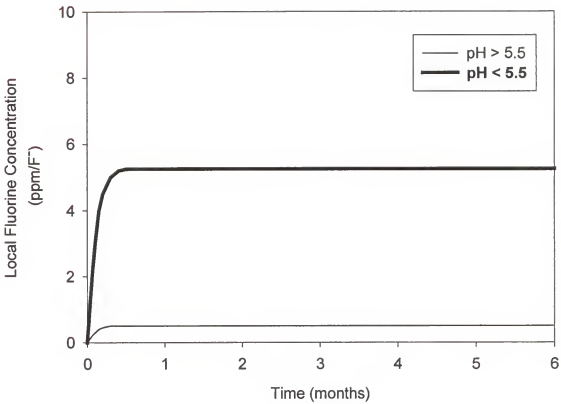


Figure 1.1: Ideal pH-sensitive self modulated fluoride release curve.

CHAPTER 2
BACKGROUND

2.1. Controlled Drug Release Technology

2.1.1. Advantages of Controlled Release Technology

Controlled drug release technology is a field which has developed rapidly in the past quarter of century. The concepts behind this technology have been systematically reviewed and described in detail (Chien, 1992a). The basic retionale for the controlled drug delivery is to alter the pharmacokinetics and pharmacodynamics of pharmacologically active moieties by using novel drug delivery systems or by modifying the molecular structure and/or physiological parameters of the drug. For each drug, there exists a therapeutic window of plasma concentration, below which therapeutic effect is insufficient, and above which undesirable or toxic side effects are elicited, as shown in Figure 2.1. In general, controlled-release drug delivery currently involves control of either the time course or location of drug delivery, while control of the time course of drug delivery is the more classical approach. Controlled drug release technology provides the following unique advantages over conventional routes of drug administration:

1) Dosage regulation, which maintains the drug concentration within the therapeutic window, without the peak of overdosing commonly associated with the traditional routes of drug administration.

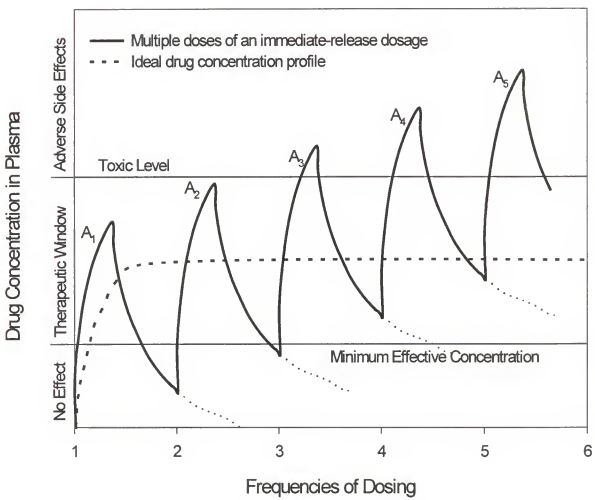


Figure 2.1: Comparison of drug concentration in plasma from a traditional route of administration and controlled release device (Chien, 1992a).

2) Prolonged duration of drug action, with reduced dose frequency and improved efficacy of drugs as well as patient compliance.

3) Localized and self-regulated drug delivery, which release a drug only upon demand at the site of action, and provide a route of targeted drug delivery with maximized therapeutic efficacy and minimized systematic toxicity.

2.1.2. Two Basic Types of Rate-Controlled Drug Release Systems

Regardless of the sophistication of the technology in use, controlled drug release devices consist of the following common structural elements:

1) Drug reservoir;

2) Rate-controlling element;

3) Energy source.

Two basic rate-controlled drug delivery systems are as follows: 1) Polymer membrane permeation-controlled drug delivery systems. In this type of controlled-release drug delivery system the drug reservoir is encapsulated inside a polymeric membrane. The polymer membrane acts as a rate-controlling element, and the drug release rate is controlled by the drug permeation through the rate-controlling membrane. 2) Polymer matrix diffusion-controlled drug delivery system. In this type of controlled-release drug delivery system the drug reservoir is homogeneously dispersed throughout a polymer matrix. The polymer matrix acts as the rate-controlling element, and the release of the drug is thus controlled by its diffusion through the rate-controlling polymer matrix.

2.1.3. Difference in Release Characteristics of Two Basic Types of Rate-Controlled Drug Release Systems

Theoretically, the controlled release of drugs from both membrane permeation and matrix diffusion controlled drug delivery systems is governed by Fick's laws of diffusion (Eqn 2.1), which defines the flux of diffusing species, J_D, across a plane surface of unit area as follows:

$$J_D = -D\frac{dC}{dx} \qquad \text{(Eqn 2.1)}$$

where D is the diffusivity of the drug molecule in a medium of solid, solution, or gas; dC/dx is the concentration gradient of the drug molecule across a diffusional path with thickness dx; and a negative sign is used to define the direction of diffusion from a region with high concentration to a region with low concentration. In these types of controlled release devices, the drug concentration gradient acts as the primary energy element for the diffusion of drug molecules. However, kinetically, the release of drug molecules from these two types of drug delivery systems is essentially controlled by two different mechanistic patterns that result from the time dependency of the diffusional flux J_D. Therefore, different physical models should be used for mechanistic analysis of the rate-controlled delivery of drugs from these basic types of controlled-release drug delivery systems.

In the case of the membrane permeation-controlled drug delivery devices, the drug concentration gradient dC/dx across a constant thickness of polymeric membrane is essentially constant and thus is invariable with time. For one-dimensional diffusion to a

plane surface, the cumulative amount of drug Q released from a unit surface area of a polymer membrane permeation-controlled drug delivery device can be described by the mathematical expression (Eqn 2.2) (Chien, 1992b):

$$Q = \frac{C_p C_s D_d D_m}{C_s D_d h_m + C_p D_m h_d} t \qquad \text{(Eqn. 2.2)}$$

where t is time, C_p is solubility of drug in polymer, C_s is concentration of drug at the solution/polymer interface, D_m and D_d are the diffusivities in the polymer membrane and in the aqueous solution, h_m and h_d are the thickness of polymer membrane and thickness of diffusion layer, respectively, in the aqueous solution. It is important to note that Equation 2.2 indicates that polymer membrane permeation-controlled drug delivery devices should yield a constant drug release profile, that is, a zero-order release profile. Zero-order release is the ideal drug release profile as indicated in Figure 2.1.

On the other hand, the concentration gradient in matrix diffusion-controlled drug delivery devices is time dependent and decreases progressively in response to the growing increase in the thickness of the diffusional path during the course of the drug release process. Similarly, for one-dimensional diffusion to a plane surface, the cumulative amount of drug Q released from a unit surface area of a polymer matrix diffusion-controlled drug delivery device can be described by the mathematical expression (Eqn. 2.3) (Chien, 1992b):

$$Q = \left[(2A - C_P)C_P D_P t\right]^{1/2} \qquad \text{(Eqn. 2.3)}$$

where, A is the initial amount of drug solid impregnated in a unit volume of polymer matrix (mg/cm^3), C_p is solubility of drug in polymer, D_p is the diffusivities of the drug in the polymer matrix. Equation 2.3 indicates that, after a short period of drug release, a matrix diffusion-controlled process becomes the predominant step in determining the rate of drug release. Compared to the polymer membrane controlled release, the significant difference of the polymer matrix controlled release is that the cumulative amount of drug release is proportional to the square root of time of release.

During the course of designing a controlled drug release device, the combination of these two basic controlled-release drug delivery systems led to membrane-matrix hybrid drug delivery systems. These hybrid systems not only provided more system parameters to achieve the desired drug release profiles, but maintained the mechanical superiority of polymer matrix diffusion-controlled drug delivery systems even after complete release of the loaded drug.

2.1.4. pH-activated Drug Delivery System

The ideal drug delivery system should provide therapeutics in response to physiological requirements, having the capacity to "sense" changes and alter the drug release process accordingly. Activation-modulated drug delivery systems is one branch of the rate-controlled drug delivery system. In this group of controlled release drug delivery systems the release of drug molecules from the delivery systems is activated by some physical, chemical, or biochemical processes. In recent years, several research

groups have been developing responsive systems that more closely resemble the normal physiological process in which the amount of drug released accords with physiological needs (Kost, 1990). The pH-activated drug delivery systems are a branch of this category. Responsive polymer systems have been investigated for self-modulated delivery of therapeutics (Kost and Langer, 1992). This type of drug delivery system permits the delivery of a drug selectively, either in the region with a selected pH range, or at the time when the environmental pH changed to the selected pH range.

Most pH-activated drug delivery systems have been based on pH-sensitive hydrogels. A hydrogel is defined as an infinite three dimensional polymeric network with a large water content, that is, more than 20 wt% (Ratner and Hoffman, 1976). Along with other biomedically favorable properties, their high, and at the same time, controllable permeability to drug molecules lays the foundation for their wide acceptance in development of controlled drug delivery systems (Mack et al., 1986). pH-sensitive hydrogels are polyelectrolyte gels usually containing ionic or ionizable pendant groups from the main polymer chains. Those pendant groups could be weakly acidic and basic groups, such as carboxylic acids, primary or substituted amines, or strong acids and bases, such as sulfonic acids and quaternary ammonium salts. Those pendant groups change ionization in response to changes of environmental pH, thus changing the properties of the gel (Kopecek et al., 1971).

For a pH-activated drug delivery system, one of the most significant changes in properties of the pH-sensitive hydrogels is the discontinuous change of the equilibrium degree of swelling upon the changes of the environmental pH (Brondsted and Kopecek, 1992). This change in equilibrium degree results in the change of the permeability of

water molecules, and is related to the change of diffusivity of drug molecules in polymeric networks as well. Furthermore, the properties of pH-sensitive hydrogels can be tailored by copolymerization with hydrophilic or hydrophobic monomers. In principle, hydrogels with the desired drug permeability, pH-sensitivity, and mechanical properties can be obtained by varying the amount and type of monomer and cross-link agent used in the copolymerization. All of these variables indicate that there exists a great potential in using pH-sensitive hydrogels for the development of a self-modulated drug delivery system.

2.1.4.1. The driving force of swelling of pH-sensitive hydrogel

Swelling of the pH-sensitive hydrogel is a balance of opposing forces. At a critical pH, ionization/dissociation occurs for the pendant weak acid/basic groups attached to the main polymer chains. This dissociation of acidic or basic groups in the gel results in the diffusion of counterions into the gel from the surrounding medium to maintain the electroneutrality of the gel. Many factors contribute to the swelling of hydrogels. First, after the ionization, there is the tendency of the polar and ionic constituents of the hydrogel to surround themselves with solvent molecules. The formation of the solvation shells of the fixed and mobile ions initiates the swelling process. The formation of the solvation shells of the ionizable groups results in a highly concentrated solution of ions inside the lightly cross-linked hydrogel, which has a tendency to dilute itself by taking up additional solvent. In the macroscopic models, this effect appears as an osmotic pressure difference between the interior of the hydrogel and the external solution, resulting in an increase in swelling described by the Donnan

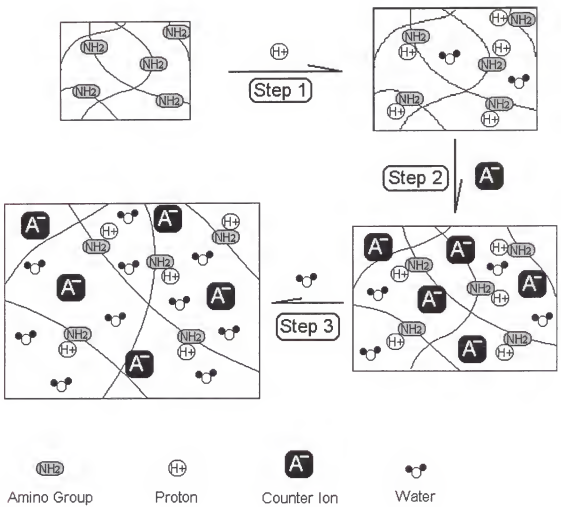
equilibrium. Along with the swelling of the hydrogels, the retraction force of the expanded polymer chain increases. At equilibrium, the difference of osmotic pressure is balanced with the retraction force of the stretched polymer network. Of course, electrostatic forces (in particular the repulsion between neighboring fixed ionic groups in the polymer chain) and interactions between the solvent and the matrix (especially if the solvent is organic) may also contribute to increases in swelling of the hydrogel. However, the difference of osmotic pressure across the surface of the hydrogels serves as the primary driving force for the swelling process, and thus relates directly to the rate and equilibrium of swelling of the hydrogel. Firestone and Siegel studied the aqueous kinetic swelling properties of a class of cross-linked hydrophobic polyamine copolymer gels based on n-alkyl esters of methacrylic acid (nAMA) and N,N-dimethylaminoethyl methacrylate (DMA) (1991). Wei and Batich studied the swelling behavior of a class of a copolymer of styrene and DMA.(Wei, 1995; Wei and Batich, 1995b). Based on these observations, a four-step mechanism for swelling of hydrophobic, ionizable gels in ionic solution can be proposed (Figure 2.2):

Step 1: Protonation of the amine groups of the gel at the critical pH.

Step 2: Transportation of the counterion into the gel due to electrostatic attraction.

Step 3: Infusion of external solvent and expansion of polymer in the vicinity of the ionized interface in order to balance the difference in osmotic pressure across the interface.

Step 4: Balance of the swelling force with the retracting force of the expanded polymer network.



Step 1: Protonation.
Step 2: Diffusion of Counterions.
Step 3: Hydration.

Figure 2.2: Schematic illustration of the swelling process of a pH-sensitive hydrogels containing amino groups.

2.1.4.2. Factors influencing the swelling of a pH-sensitive hydrogel

Understanding the factors influencing the swelling of pH-sensitive hydrogel is extremely important in the course of designing a self-modulated controlled drug release device. Many factors, including both intrinsic factors and extrinsic factors, influence the swelling behavior of the pH-sensitive hydrogel. These intrinsic factors include the type of charge of the ionic monomer, pK_a of the ionizable group, degree of ionization, concentration of ionizable monomer in the polymeric network, crosslink density, and hydrophilicity/hydrophobicity of the polymer (as for uncharged gels). The extrinsic factors are pH, ionic strength and concentration of the swelling solution, pK_a of the acid, valence and hydrophilic/lipophilic property of the counterions in buffer solutions, and temperature. Table 2.1. summarizes these factors which influence the equilibrium swelling extent of the hydrogel.

The type of charge of the ionic monomer is the primary factor effecting the pH-sensitivity of the gel. An anionic/acidic hydrogel (such as gels containing $-SO_3H$, or -COOH groups) will be ionized at high pH (Khare and Peppas, 1995), thus, the extent of swelling will increase at high pH (Kou et al., 1988). In contrast, a cationic/basic hydrogel will be protonated at low pH, therefore, the hydrogel has the opposite pH-dependence of swelling (Siegel and Firestone, 1988).

The pK_a of the ionizable group was shown to influence the swelling-transition pH of the pH-sensitive hydrogel. An increase in the pK_a of an acidic monomer will lead to a higher swelling transition pH (Brannon-Peppas and Peppas, 1991). It was shown that the swelling response to pH was very sensitive at a pH close to the pK_a of the ionizable group

Table 2.1: Factors influencing equilibrium swelling of polyelectrolyte hydrogels.

Type	Factor	Effect
Intrinsic factors	Charge of ionizable monomer	Acidic: pH ↑ ⇒ ionization ↑ Basic: pH ↓ ⇒ ionization ↑
	pK_a of ionic monomer	pK_a ↑ ⇒ swelling onset pH ↑
	Degree of ionization	Ionization ↑ ⇒ swelling ↑
	Concentration of ionizable monomer	Concentration↑ ⇒ swelling ↑
	Crosslink density	Density ↑ ⇒ swelling ↓
	Hydrophilicity of polymer backbone	Hydrophilicity ↑ ⇒ swelling ↑
Extrinsic factors	pH	Acidic gel: pH ↑ ⇒ swelling ↑ Basic gel: pH ↓ ⇒ swelling ↑
	Ionic Strength	Ionic strength ↑ ⇒ swelling ↓ or ↑
	Counterion	Effect depends on species (Salting-in/salting-out and hydrophilic/lipophilic properties)
	Valence of counterion	Valence ↑ ⇒ swelling ↓

of the hydrogel. Brannon-Peppas and Peppas applied Florey-Rheiner theory, rubber elasticity theory, and ionic interactions to the swelling of a highly swellable anionic pH sensitive hydrogel. They found that the swelling transition of the hydrogel relates closely to the pK_a of the pH-sensitive monomer. Theoretically, at the pH value equivalent to the pK_a of the pH sensitive monomer, the swelling extent is predicted to reach 50% of its maximum value.

The concentration of ionizable monomer in the hydrogel and degree of ionization of the hydrogel influence equilibrium swelling extent and swelling transition pH of the hydrogel. Either an increase in ionizable monomer in the hydrogel, or an increase in the degree of ionization of the hydrogel, results in an increase in osmotic pressure inside the gel; this further leads to an increasing extent of swelling (Kopecek et al., 1971; Brannon-Peppas and Peppas, 1991). Batich and coworkers reported that the equilibrium swelling extent of a copolymer of styrene with 4-(or 2-) (vinyl pyridine) increased in copolymers with a high vinyl pyridine content. Also, the swelling transition pH of the copolymer shifted to a low pH as the vinyl pyridine content in the copolymer increased (Figure 2.3) (Batich et al., 1993). Wei found a similar trend in the pH-sensitive release of a dye from a copolymer of styrene (St) with N,N-dimethylaminoethyl methacrylate (DMA) as shown in Figure 2.4 (Wei, 1995). The shift of the swelling transition pH of the hydrogel could be attributed to the electrostatic interaction of the adjacent pendant ionizable groups along the chain. Because of this interaction, the apparent pK_a of the ionizable groups depends on the degree of ionization and changes with pH.

An increase in crosslink density results in a decreasing equilibrium swelling extent of the hydrogel. Batich and coworkers reported that an increase in crosslink

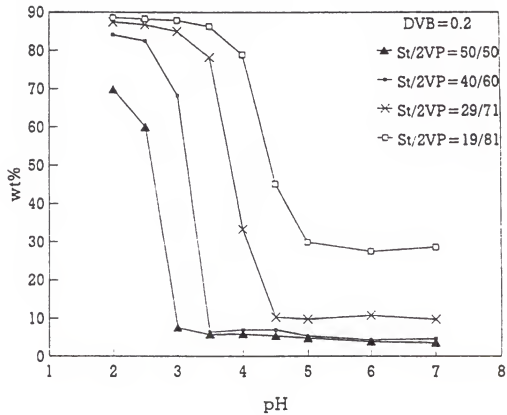


Figure 2.3: Effect of 2VP content in copolymer on the swelling onset pH of St/2VP/DVB copolymer. Water content (wt%) at equilibrium was measured in 0.05 M citric buffer solution (I=0.3 M) at room temperature (Batich et al., 1993).

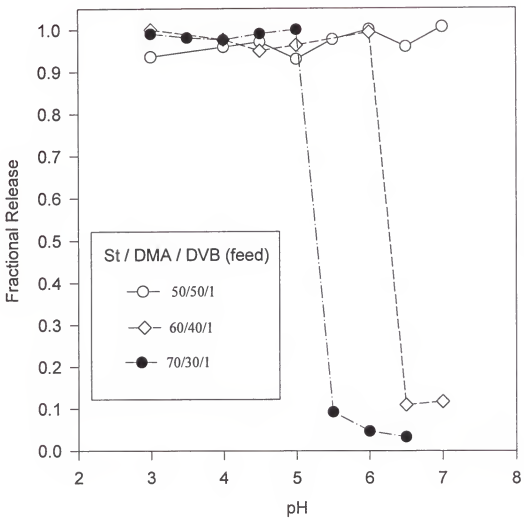


Figure 2.4: Effect of DMA content in copolymer on the release onset pH from St/DMA/DVB copolymer. Fractional release of 9-aminoacridine at equilibrium was measured in 0.05 M citric buffer solution (I=0.3 M) at room temperature (Wei and Batich, 1995a).

density in a styrene-co-vinyl pyridine hydrogel results in a decrease in the equilibrium swelling extent without influence on swelling transition pH (see Figure 2.5) (Batich et al., 1993). Accordingly, in the study of the pH-sensitive release of a dye, Wei found that release onset pH is independent of the change in crosslink density of St/DMA/DVB copolymer (see Figure 2.6). A modest increase in crosslink density (from 1 to 2%) of the hydrogel has a modest effect on reducing the dye release rate (see Figure 2.7) (Wei, 1995). Further increase in crosslink density of the copolymer matrix may compromise the loading level of the releasing agent. Therefore incorporation with additional diffusion control layer may be necessary to regulate drug release rate.

The hydrophilic/lipophilic balance of the hydrogel influences equilibrium swelling extent of the hydrogel. Siegel and Firestone found that increasing the hydrophobicity of poly(n-alkyl methacrylate-co-N,N-dimethylaminoethyl methacrylate) hydrogel by copolymerizing with more hydrophobic n-alkyl methacrylates would decrease the pH-sensitivity of the hydrogel (Siegel and Firestone, 1988). Similarly, the hydrophilic/lipophilic property of the counterions in the buffer solution also influences the swelling behavior of the pH-sensitive hydrogel significantly. Wei and Batich investigated the hydrophilicity and lipophilicity of the counterions in various buffer solutions on the swelling behavior of poly(styrene-co-N,N-dimethylaminoethyl methacrylate) hydrogels (Wei, 1995; Wei and Batich, 1995b). To eliminate the possible influence of both pK_a of the acids and composition of buffer on the swelling behavior of the hydrogel, three acids with similar pK_a value (therefore, at a certain pH, the degree of dissociation of the acids in buffer solution are very similar) and different water solubility (which indicates the hydrophilicity of the acids) and partition coefficients (which relates

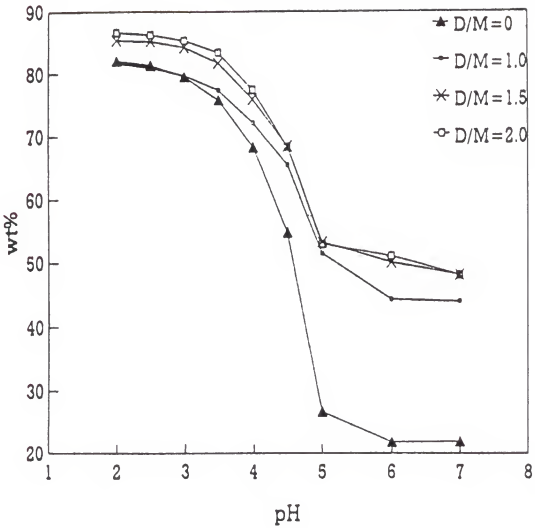


Figure 2.5: Effect of crosslink density in copolymer on the swelling onset pH and equilibrium swelling extent of St/2VP/DVB copolymer. Water content (wt%) at equilibrium was measured in 0.05 M citric buffer solution (I=0.3 M) at room temperature (Batich et al., 1993).

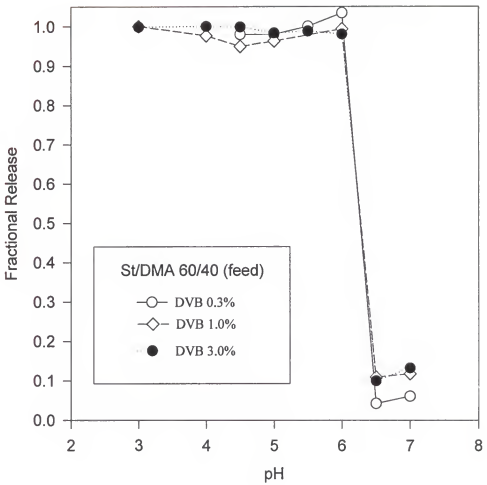


Figure 2.6: Effect of crosslink density of copolymer on the release onset pH from St/DMA/DVB copolymer. Fractional release of 9-aminoacridine at equilibrium was measured in 0.05 M citric buffer solution (I=0.3 M) at room temperature (Wei and Batich, 1995a).

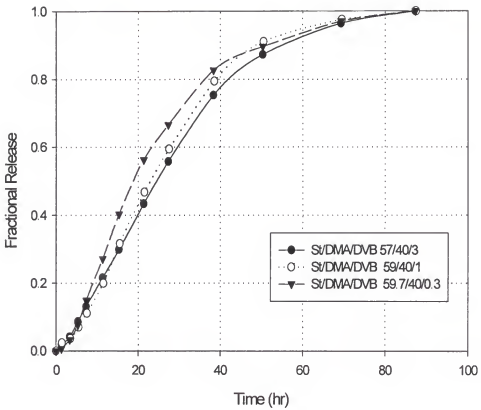


Figure 2.7: Effect of crosslink density on fractional release of 9-aminoacridine from St/DMA/DVB microspheres in 0.05 M citric buffers (I=0.3 M) at pH 5.0.

to the lipophilicity of the acids) were selected in the study. These acids were acetic acid, n-butyric acid, and phenylacetic acid . The pK_a, water solubility, and partition coefficient of acetic, n-butyric, and phenylacetic are listed in Table 2.2. Wei and Batich found a marked difference in both swelling rate and equilibrium swelling extent of the hydrogel in these buffer systems as shown in Table 2.3 and Table 2.4 (Wei, 1995; Wei and Batich, 1995b). In acetic and butyric buffer, the equilibrium swelling extent (Q_{eq}) of the hydrogel were comparable at all pH's studied, while the equilibrium swelling extent in phenylacetic acid was consistently lower than that observed in acetic and butyric buffers. A comparison of the swelling kinetics, under the same experimental conditions, showed that the time needed to reach the equilibrium swelling state (t_{eq}) was reduced significantly in the more lipophilic buffer, such as phenylacetic acid. This phenomenon can be attributed to the combined influence of lipophilicity and hydrophilicity of the buffer acid: 1) Buffer acids with high lipophilicity penetrate the hydrophobic region of the hydrogel easily. After this penetration, the acid molecules serve as a plasticizer for the copolymer network, which enhances the mobility of the polymer chains, and consequently, swelling rate of the hydrogel. 2) Penetration of the acid with high hydrophobicity increases the total hydrophobicity of the hydrogel, which leads to less water uptake, and thus a lower equilibrium swelling extent. These findings indicate that, when designing a pH-sensitive swelling controlled release device, reliable *in vitro* test results could be obtained only if there is a careful match between buffer composition and physiological conditions.

Buffer concentration and ionic strength affect the swelling of pH-sensitive hydrogels (Siegel and Firestone, 1988; Siegel et al., 1991; Kwon, 1991). As acid concentration or ionic strength of the buffer increases, the degree of swelling decreases.

Table 2.2: pK_a, water solubility, and partition coefficient (P) of acetic acid, n-butyric acid, and phenolacetic acid.

	K_a	Solubility (g/100 ml H_2O)	logP (Octanol/Water)
Acetic acid	1.76×10^{-5}	∞	-3.4
Butyric acid	1.54×10^{-5}	∞	0.67
Phenylacetic acid	4.96×10^{-5}	1.78	1.99

Table 2.3: Time to reach equilibrium swelling (t_{eq}) for St/DMA/DVB 59/50/1 copolymer in 0.1 M acetic, butyric and phenylacetic buffer solution.

	t_{eq} (hr)		
pH	Acetic	Butyric	Phenylacetic
4.5	<6	<3	<3
5.0	≈340	<11	<6
5.5	≈2000	≈500	<16
6.0	>2200	N/A	<100

Table 2.4: Equilibrium swelling extent (Q_{eq}) for St/DMA/DVB 59/50/1 copolymer in 0.1 M acetic, butyric and phenylacetic buffer solution.

	Q_{eq}		
pH	Acetic	Butyric	Phenylacetic
4.5	3.06	3.27	1.24
5.0	3.33	3.35	1.10
5.5	3.02	3.22	1.56
6.0	0.55	N/A	1.62

This is because, as the concentration of ions outside the hydrogel increases, the concentration gradient across the surface of the gel is reduced. Therefore, the osmotic pressure inside the gel, the primary swelling force, decreases with the increase of concentration (or ionic strength) of the buffer solution.

The pH of the buffer solution influences the ionization of the pendant weak acidic/basic groups in the pH-sensitive hydrogels directly. Siegel and coworkers studied the swelling behavior of the MMA/DMA copolymer in acetic, methoxyacetic, and cholroacetic buffer solutions (Siegel et al., 1992). They found that, at fixed pH, buffer concentration, and ionic strength, the swelling rate varies in different buffer systems, while the equilibrium swelling extent are virtually the same. The author concluded that pK_a of the acid in buffer may also influence the swelling behavior of the pH-sensitive hydrogel by varying the concentration of the conjugated acid form in the buffer solution.

The type of co-ion did not influence the swelling behavior of pH-sensitive hydrogels, since co-ions do not very easily permeate through a swollen gel of the same charge. However, the nature of the counterion has been shown to influence the swelling behavior of the hydrogel significantly (Grignon and Scallan, 1980; Ohmine and Tanaka, 1982; Nakano et al., 1990; Vasheghani-Farahani et al., 1990). A buffer containing multivalent counterions decreases the degree of swelling of the gel. One multivalent ion is able to neutralize several counter charges. Therefore, compared to monovalent counterions, less multivalent counterions will be needed to neutralize the same amount of opposite charges inside the ionized hydrogel. Thus, the osmotic pressure and the degree of swelling of the gel will be decreased. In addition, multivalent counterions may also

serve as ionic crosslinks between ionized polymer chains, which further restricts the expanding of the network of the hydrogel.

2.1.4.3. Special consideration for design of pH-sensitive drug delivery system

As discussed in the previous section, many factors influence the swelling behavior of pH-sensitive hydrogels. The pH-sensitive swelling behavior of the gel matrix relates closely to the controlled drug release behavior. For design of a controlled release device, several other factors that influence the swelling behavior of the pH-sensitive hydrogel should be included for consideration. These factors are:

1) Drug loading level in the copolymer. High drug loading levels, particularly for those highly ionic drugs, may significantly increase the osmotic pressure inside the hydrogel after ionization. Therefore, the swelling behavior of the polymer matrix may altered by an increasing degree of swelling and by a shifting of pH-sensitivity of the gel matrix.

2) Physical/chemical properties of the loaded drugs, such as molar mass, solubility, hydrophilicity/lipophilicity of the drug molecules, partition coefficient, etc. The physical/chemical properties of the loaded drugs may alter the total hydrophilic/hydrophobic balance of the originally designed polymer matrix, which results in a change of degree of swelling and rate of drug release. This observation was explained by the fact that some species are water structure breakers that increase swelling of the gel, whereas others are structure makers that decrease swelling of the gel (Siegel et al., 1991). Also, depending on the compatibility between the drug molecules and the polymeric network, the degree of swelling of the hydrogel may be altered from its

original design, due to the salting-out or salting-in effect of the loaded drug molecules (Refojo, 1967).

3) Interaction between drug and molecules dissolved in the buffer solution. For example, the formation of a less soluble form of drug derivative by interaction between the loaded drug and molecules in the buffer solution may sharply reduce the osmotic pressure inside gel. Furthermore, formation of a precipitate may block micro-channels for diffusion, which leads to a decrease in diffusivity and thus the rate of release.

Due to the multifunctional influence on the swelling behavior of the pH-sensitive matrix, an *in vitro* controlled release study is essential for successful design of a drug delivery system. To ensure reliable result from an *in vitro* controlled drug release study, the buffer system should closely resemble the physiological values of the specific site where the controlled release device is intended to be used.

2.2. Etiology of Caries

2.2.1. Primary Factors for Dental Caries and Strategy for Caries Prevention

Dental caries is a multifactorial disease. Interaction between three primary factors is essential for the initiation and progression of dental caries: 1) susceptible host, (primarily the saliva and teeth), 2) a microflora with cariogenic potential, and 3) a suitable local substrate, or diet, to meet the requirements of the pathogenic flora. Diagrammatically, these factors can be portrayed as three overlapping circles as shown in Figure 2.8 (Nikiforuk, 1985). In addition, a fourth factor - time - must be considered in any discussion of the etiology of dental caries. For caries to occur, conditions within

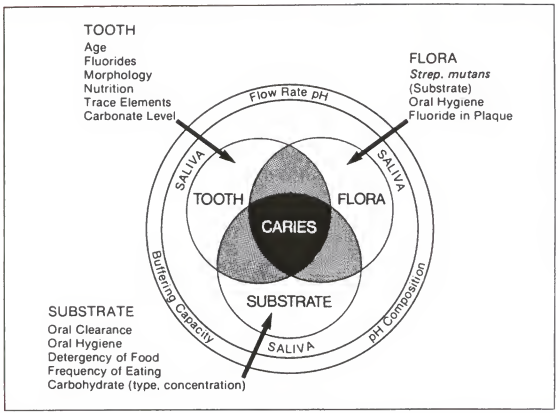


Figure 2.8: Diagrammatic representation of the interplay between primary and secondary factors in caries etiology. (Nikiforuk, 1985).

each of these factors must be favorable. In other words, caries requires a susceptible host, a cariogenic oral flora, and a suitable substrate which must be present for a sufficient length of time. Conversely, the strategy for caries prevention is based on the same factors: 1) increase the resistance of the host by fluoride therapy, occlusal sealants, and immunization; 2) lower the numbers of microorganisms in contact with the teeth by plaque control; 3) modify the substrate by selecting noncariogenic food stuffs; and 4) reduce the time that the substrate is in the mouth by limiting the frequency of intake.

2.2.2. Interaction between the Primary Factors of Dental Caries

During the progression of the dental caries, tooth is the target tissue destroyed in the dental caries process. Obviously, the resistance of the tooth is important since this determines the overall effect of the carious attack. The cariogenic oral flora which is localized to specific sites on teeth is the agent that produces and secretes the chemical substances (such as organic acids, chelating agents and proteolytic enzymes) that cause the destruction of the inorganic components and the subsequent breakdown of the organic moieties in enamel and dentin. The local substrate provides the nutritional and energy requirements for the oral microflora, thereby permitting them to colonize, grow, and metabolize on selective surfaces of teeth. Of the metabolic endproducts implicated in the dental caries process, organic acids are most important in mineral dissolution (Nikiforuk, 1985). The pH at which any particular saliva ceases to be saturated with calcium and phosphate is referred to as the "critical pH"; below this value, the inorganic material of the tooth may dissolve. The critical pH varies according to the calcium and phosphate concentration, but it is usually about 5.5 (Newbrun, 1983). Numerous animal studies

clarify the role of microorganisms in dental caries. Important summary points are as follows:

1) Microorganisms are a prerequisite for dental caries initiation.
2) A single type of organism is capable of inducing caries.
3) The ability of the microorganism to produce acid plays a major role in the dental caries process.

2.2.3. Cariogenic Microflora

Many aspects of oral bacterial ecology have been reviewed in detail in terms of the dynamic relationship among the dental plaque microbiota, dietary carbohydrate, saliva, and the pH-lowering (van Houte, 1994; Gibbons and van Houte, 1973, 1975; Socransky and Manganiello, 1971). Although there are different opinions as to how and which microorganisms produce carious lesions, it is uniformly agreed that caries cannot occur without the presence of microorganisms. In other words, " a clean tooth never decays". The classical germfree animal studies of Orland et al. firmly established the principle that dental caries is a bacterial infection (Orland et al., 1954, 1955). Several organisms have been found capable of inducing carious lesions in the gnotobiotic rats. These cariogenic organisms include *Streptococcus mutans, Streptococcus salivarius strain, Streptococcus milleri strain, Streptococcus sanguis, Peptostreptococcus intermedius*, *Lactobacillus acidophilus* and *Lactobacillus casei strain*, *Acitinomyces viscosus*, and *Acitinomyces naeslundii* (Newbrun, 1983). However, not all of these organisms are equally virulent. Although it is not known exactly what determines the cariogenicity of a microorganism, it is generally accepted that a variety of *Streptococcus*

mutans that produces dextrans (an extracellular polysaccharide containing glucose units) from sucrose plays a significant role in the induction of dental caries in pits and fissures, on smooth surfaces, and on root surfaces of the tooth (Nikiforuk, 1985; Loesche et al., 1975; Gibbons and van Houte, 1975). *In vivo* and *In vitro* studies have shown that *Streptococcus mutans*, and to a lesser extent *Streptococcus sobrinus*, are main microorganisms responsible for dental caries (Hamada and Slade, 1980; Loesche, 1986).

2.2.4. Acid Profiles of Dental Caries

Organic acids produced by micro-organisms play an important role not only in demineralizaing the inorganic minerals, but also in enhancing bacterial penetration (Michelich et al., 1980) and proteolytic destruction of organic matrix of the tooth (Katz et al., 1987; Goldberg and Keil, 1989). In vitro studies of human dental plaque show that caries activity is associated with a higher rate of acid production (van Houte et al., 1996; Minah and Loesche, 1977) and faster pH drop (Igarashi et al., 1987). Carious dentin contains a variety of organic acids, such as lactate, acetate, propionate, butyrate, valerate and caproate (Hojo et al., 1994; 1991). Although lactate, acetate and propionate are major acids, their proportions, i.e. the acid profile, vary markedly depending on the progression stage of caries. Hojo and coworker studied the acid profiles of active and arrested carious lesions in dentin. They found that clinical classification of dentin caries clearly relates to both the local pH and acid profiles (Hojo et al., 1994). They found that the pH of carious dentin was distinctly lower than that of sound dentin. Dentin taken from active lesions showed a mean pH of 4.9, and the dominant acid was lactate (mean percentage, 88.2 mol%). In contrast, carious dentin from arrested lesions showed a

higher pH, 5.7, and the dominant acids were acetate and propionate (mean percentage, 64.0 mol% and 18.2 mol%, respectively). The variation in pH and acid profiles relates to micro-organism activity in dentin caries etiology. The environment of an active carious dentin can be characterized by the presence of a large number of lactic acid producing bacteria such as lactobacilli and streptococci (Hahn et al., 1991; Kneist et al., 1989). When sugar is available, such bacteria can be expected to produce lactic acid and, consequently, to promote local acidity. Iwami et al (1992) reported that *Streptococcus mutans* also produces more lactate at acidic than at neutral pH. As shown in Table 2.5, a dominant lactic acid presence in carious dentin serves as a distinct marker for active caries.

2.3. Function of Fluoride on Caries Prevention

No other factor in preventive dentistry has been as thoroughly documented as the cariostatic benefits of the fluoride ion (Margolis and Moreno, 1990). In 1956, the Surgeon General of the United States officially stated that dental caries prevention and safety factors of controlled water fluoridation place it among the most conclusively proven public health benefits known. Leverett et al. (1987) showed that individuals with a higher salivary fluoride content had lower caries levels than individuals with lower salivary fluoride levels. Shields et al. reported that zero-caries subjects from both fluoridated and non-fluoridated communities had salivary fluoride levels of 0.04 ppm or greater, whereas high-caries subjects from both fluoridated and non-fluoridated communities had salivary fluoride levels of 0.02 ppm or less (1987). In addition to

Table 2.5: Percentage of organic acids in active and arrested carious dentin (Hojo et al., 1994).

Type of Acids	Active (n=15)	Arrested (n=14)
Lactate	88.2 ± 8.3 mol%	7.5 ± 6.5 mol%
Acetate	9.6 ± 5.9 mol%	64.0 ± 14.4 mol%
Propionate	1.2 ± 1.1 mol%	18.2 ± 9.2 mol%
i-Butyrate	not detected	0.5 ± 1.7 mol%
n-Butyrate	0.6 ± 0.9 mol%	4.9 ± 5.4 mol%
i-Valerate	0.1 ± 0.1 mol%	0.9 ± 1.4 mol%
n-Valerate	0.1 ± 0.4 mol%	1.7 ± 2.7 mol%
i-Caproate	not detected	0.3 ± 0.7 mol%
n-Caproate	0.2 ± 0.7 mol%	2.0 ± 4.1 mol%

n = sample number.

systemic administration during the period of tooth formation and mineralization, topical application of fluoride are without a doubt the most important way of using fluoride in caries prevention. In particular, the widespread use of fluoride-containing toothpaste is thought to be a major factor in the worldwide decrease in dental caries (WHO, 1994).

However, when it comes to understanding exactly how fluoride works in reducing tooth decay, several different hypotheses have been proposed for both dentin and enamel. These include the following mechanisms (Newbrun, 1986):

1) Action on the hydroxyapatite of the enamel per se:

   a) decreasing its solubility through conversion of hydroxyapatite to fluorapatite [$Ca_5(PO_4)_3F$].

b) remineralizing calcium-depleted carious lesion with deposition of a mixture of fluoride salts.

c) improving its crystallinity

2) Action on the bacterial activity:

a) inhibiting enzymes.

b) suppressing cariogenic flora.

3) Action on the tooth surface:

a) desorbing proteins and/or bacteria.

b) lowering the surface free energy.

c) alteration of tooth surface morphology.

It is generally accepted that mechanisms 1 and 2 play a major role in the beneficial effects of fluoride, but the extent to which these mechanisms operate depends on prevailing conditions, such as the concentration of fluoride available. Brown and coworkers studied the fluoride concentration on the surface layer of fluoride treated enamel (1977). They found a sharp gradient of fluoride concentration, with highest concentrations (up to 5000 ppm) in the outermost micrometers and a much lower fluoride content in the bulk of the enamel. The substitution of hydroxyapatite by fluorapatite at the surface of the tooth causes a significant reduction in solubility and could contribute to the cariostatic effect of fluoride. It is believed that, even a low concentration of fluoride ions would tend to convert the surfaces of hydroxyapatite crystals to a thin layer of fluorapatite, which makes them behave as though they were fluorapatite.

The role of fluoride in promoting remineralization of carious dentin (Mellberg and Mallon, 1984) is also important in its caries-protective action. The ability of fluoride to

promote the formation of fluorapatite helps in the repair of defective apatite structure. In the presence of fluoride, even at low concentrations (2.5-5 ppm), calcium-deficient carious lesions preferentially reacquire calcium from solutions containing calcium and phosphate (Ingram and Nash, 1980). Low concentrations of fluoride ion greatly enhance the degree and rate of remineralization process (Silverstone, 1983). When carious lesions remineralize in the presence of fluoride, the average size of hydroxyapatite crystals increases significantly. Since this provides a less porous structure and a lower surface-area-to-volume ratio, this would reduce any subsequent dissolution of the tooth structure.

The inhibitory effect of fluoride on bacterial activity has been established (Menaker, 1980; Eriscon, 1977). The cariostatic effect of fluoride could be attributed to inhibiting enzymes and suppressing cariogenic flora as well. *Streptococcus mutans* is the most virulent oral streptococcus in terms of cariogenicity. It consistently initiates decay when inoculated into a susceptible host and is found in high proportions in the plaque on carious tooth surfaces. At low levels of fluoride (20 ppm or less), there is little evidence of alteration in the formation, microbiological composition, or acid production of oral microflora. At these low levels, most bacteria are either insensitive to fluoride or able to adapt to its presence (Hoeven and Franken, 1984). The high concentration of fluoride used in topical applications is bactericidal, which suppresses the oral cryogenic flora. However, many fluoride preparations (such as gels or varnishes) containing high fluoride concentrations in the oral cavity may be toxic, especially in children after accidental swallowing (LeCompte and Whitford, 1985). In contrast, even at a low concentration, fluoride is effective at inhibiting various enzymes which are involved in the metabolism of the oral microflora. Organic acids produced as the byproducts of metabolic activity of

oral microflora play important roles in initiation and progression of dental caries (Hojo et al., 1994). The minimum fluoride concentration found by various investigators to inhibit acid production in bacterial cultures has ranged from 2 to19 ppm F (Wiseman, 1970). Enolase, an important enzyme in glycolysis and acid formation by bacterial fermentation, shows about 50% inhibition at 0.5 ppm F (Newbrun, 1986).

2.4. Efficacy of Fluoride Delivery

2.4.1. Slow Release of Fluoride

A low and consistent level of local fluoride concentration has been related to remineralization of early carious lesions. Most of the conventional methods of fluoride therapy, including both systemic and topical administration, rely on a measure of compliance by patients for maximum efficacy (Fejerskov et al., 1981). One of the biggest advantages of slow-release of fluoride is compliance by patients. Slow-release formulations reduce the frequency of dosing, thereby simplifying and improving the probability of compliance. In addition, they are capable of maintaining effective levels of fluoride at the intended site of action without causing systematic adverse effects. Mellberg and coworkers studied the duration of elevated levels of the fluoride at the enamel surface after a single topical fluoride treatment (1966). They found that two thirds of fluoride could be lost from the enamel one day after a topical treatment with stannous fluoride. Williams and coworkers reported that prolonged low levels of fluoride could provide greater fluoride uptake efficiency than multiple applications of the short-lived elevated levels of fluoride obtained by conventional topical fluoride administration

(1982). Mirth and coworkers compared the topical and systemic anti-caries effect of fluoride in rats (1985). Studies have shown that systemic fluoride has little or no effect on the incidence of caries in the rat when administered at a rate comparable to the rate at which topical fluoride was administered by the controlled release fluoride reservoir. In other words, the cariostatic effects of the fluoride-releasing device were mainly from topical effects of local fluoride exposure, not systemic effects. Presumably, this occurs because the controlled release device provides a continuous fluoride exposure, whereas the systemic administration provides only intermittent fluoride exposure. The slow release fluoride delivery systems have had significant potential as effective anticaries agents (Mirth et al., 1983), because of the ability of controlled release formulations to provide a continuous, localized release of fluoride. Fluoride application by slow release methods has been suggested to prevent decay (Bottenberg et al., 1991; Mirth et al., 1985) and to remineralize incipient enamel lesions (Corpron et al., 1991), such as those so-called "white-spot lesions" seen in patients when removing orthodontic bands. A variety of dosage forms, including both polymer membrane permeation-controlled drug delivery systems and polymer matrix diffusion-controlled drug delivery systems, were reviewed by McKnight Hanes and Hanes (1986), and more recently by Toumba and Curzon (1993).

2.4.2. Recurrent Caries and Fluoride

Recurrent caries is the secondary carious attack beneath or around a dental restorations. Because of its location, not only is the diagnosis of recurrent caries difficult, but also the common preventive procedures (such as topical fluoride therapy provided by

fluoride gels or mouth rinse fluid) are ineffective for prevention of this type of caries. Currently, the clinician has no simple and reliable way of detecting the presence of early recurrent caries (Kidd, 1990). Recurrent caries is still the primary cause for replacement of dental restorations (Mjör, 1997, 1981; Qvist et al., 1986a, 1986b).

Like primary caries, recurrent caries around restorations is caused by plaque action and/or the action of acids in the oral cavity. It has been well documented that the incidence and severity of secondary caries are reduced in adjacent tooth surfaces around restorations that release fluoride (Phillips, 1988; Hicks et al., 1986; Volker et al., 1944). Silicate restorative materials and glass-ionomer cement contain large mounts of fluoride (about 15-20 percent). This explains the documented elevated concentration of fluoride in tooth structures surrounding such restorations (Forsten et al., 1976). A slow release of flouride from a restoration is effective against potential secondary caries (Arends et al., 1990). This is because the local release of fluoride on the tooth/restoration interface not only promotes the remineralization of the carious lesion, but also provides a locally high fluoride concentration to suppress the bacterial activity without causing systemic toxicity.

2.4.3. Glass Ionomer Cement and Its Limitations

Glass ionomer cements were developed in the early 1970's (Wilson and Kent, 1972). Recent developments in the manufacturing of light-cured glass ionomer composite resin hybrid cements showed the potential advantage of their ability to release fluoride (Swartz et al., 1984), thereby increasing the caries resistance of the tooth structure surrounding the restoration. Recently, glass ionomer cements have been explored for many applications, based on its effect of fluoride release. Some examples of

these applications are light-cured bases and liners (DeSchepper et al., 1990, Strickland et al., 1990), orthodontic bonding materials (Chadwick and Gordon, 1995a, 1995b; Wiltshire and Janse van Rensburg, 1995; Nigel, 1990), crown foundations (Knibbs, 1988; van de Voorde et al., 1988), permanent restorations (Swift, Jr. et al., 1990), and other removable appliances (Cooley and McCourt, 1991). Studies using glass ionomer cement to bond orthodontic brackets have shown that this material protected against demineralization of the enamel underneath and also 1 mm around the orthodontic bracket *in vitro* (Valk and Davidson, 1987). Fluoride release from glass ionomer liners under amalgam restorations may also be useful in preventing recurrent caries (Olsen et al., 1989; Hicks et al., 1986; Swartz et al., 1984;). Several glass ionomer cement liners for use under amalgam and composite restorations have become commercially available. These include: Vitrabond Light Cure Glass Ionomer Liner/Base (3M Company, St. Paul, MN, USA); Light Cured Zionomer Powder/Liquid; Light Cured Zionomer Paste/Paste(Den-Mat Corp., Santa Maria, CA, USA). In addition, a fluoride containing adhesive dentin bonding base, VLC TimeLine Base/Liner (L.D. Caulk Co., Milford, DE, USA) and a glass ionomer dentin substitute, GC Dentin Cement (GC Dental Industrial Corp., Tokyo, Japan) have been introduced. However, different research groups (McCourt, 1990,; Hatibovic-Kofman and Koch, 1991; Friedl et al., 1997) observed a burst release of fluoride from light-cured glass ionomers within the first and second days after curing, with the fluoride release rate decreasing significantly, as shown in Table 2.6 and Figure 2.9. Most recurrent caries occurs at least several months after the initial restoration procedure. Therefore this burst of fluoride release may compromise its effectiveness against the recurrent caries. Recently, Friedl and coworkers investigated the

influence of fluoride released from several resin-modified glass ionomer cements on *Streptococcus mutans* growth *in vitro* (1997). A noticeable suppression of *S. mutans* growth was observed only during the first 2 weeks (see Figure 2.10). In addtion, insufficient bonding stength and poor marginal sealing effectiveness (Sidhu, 1992; Brown et al., 1993) of light-cured glass ionomer cement liner limit its practical value against recurrent caries. Recently, the effectiveness of glass iononmer material on prevention of recurrent caries has been challenged in a clinic study (Mjör, 1997).

Table 2.6: Fluoride release from several commercial glass ionomer materials. Fluoride concentration (ppm) was measured in 2 ml saline from a cylindrical glass ionomer specimen (3 mm diameter, 6 mm hight) (Friedl et al., 1997).

	Elution time			
Material	48 h	14 days	90 days	180 days
Ketac-Fil	27.3 ± 1.4	5.4 ± 0.7	2.3 ± 0.3	1.7 ± 0.3
Ketac-Silver	6.2 ± 1.6	1.8 ± 0.1	0.8 ± 0.1	0.6 ± 0.1
Photac-Fil	29.3 ± 2.0	6.2 ± 0.1	1.9 ± 0.1	1.4 ±0.1
Fuji II LC	27.5 ± 0.8	5.3 ± 0.3	2.0 ± 0.1	1.5 ±0.1
Vitremer	25.3 ±0.8	6.2 ± 0.4	2.2 ± 0.1	1.7 ±0.1
Dyract	7.4 ± 0.2	1.7 ± 0.0	1.0 ± 0.1	0.9 ±0.0

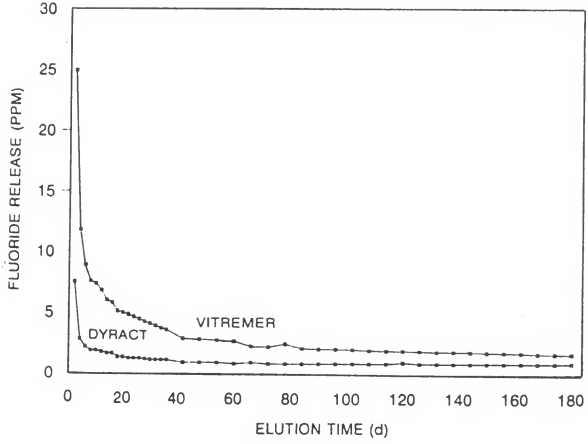


Figure 2.9: In vitro fluoride release curves from two commercial available glass-ionomer materials. Fluoride concentration was measured in 2 ml saline from a cylindrical glass ionomer specimen (3 mm diameter, 6 mm hight). (Friedl et al., 1997).

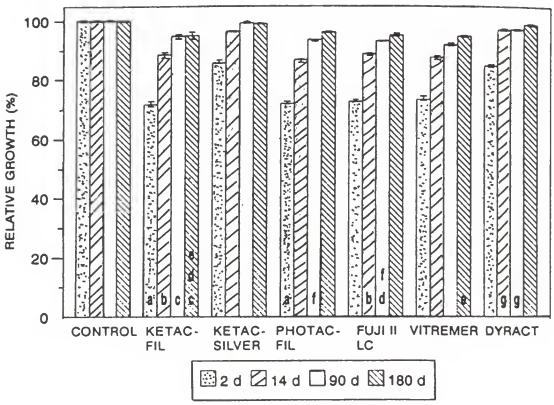


Figure 2.10: Efficacy of several fluoride releasing glass ionomer cements on suppressing *Streptococcus mutans* growth *in vitro* (Friedl et al., 1997).

2.5. Ideal Fluoride Release Device and Goal of Current Research

The goal of this research was to design and test a pH-sensitive fluoride controlled release device for localized fluoride delivery beneath the dental restorations. This device contains fluoride loaded pH-sensitive polymeric microspheres and adhesive resins. As the environmental pH drops below the critical pH for dissolution of dentine (about 5.5), the loaded fluoride will start to release from the device. To maximize the therapeutic value of the fluoride, the fluoride release rate should be controllable to maintain a desired release rate and profile. Figure 1.1 shows the ideal fluoride release profile of the device. In addition, this device can not swell extensively during the course of fluoride release, since the extensive swelling of the liner material beneath the restoration may cause discomfort to the patient or even physical damage to the teeth structure and restoration. This device should also maintain superior physical integrity and provide sufficient bonding between dentine and restorative materials, before and after the fluoride release. Therefore, the more specific goals of this study are

1) develop an efficient fluoride loading technique.
2) investigate the feasibility of pH-sensitive fluoride release from pH-sensitive copolymer in lactic buffers.
3) investigate the permeability of the adhesive resin as a regulator of fluoride release.
4) investigate the physical and mechanical properties of the controlled release device.

CHAPTER 3
EXPERIMENTAL OUTLINE

3.1. Material Selection

To maintain the mechanical superiority after complete release of fluoride, we decided to use a two-element membrane-matrix hybrid drug delivery system to approach the goals of the current research proposed in Chapter 2. The controlled release system developed in this study contains fluoride loaded pH-sensitive microspheres and a bonding adhesive layer (See figure 3.1). Fluoride loaded pH-sensitive microspheres serve as the core for self-modulated fluoride release at the desired pH. The bonding adhesive layer with balanced hydrophilicity and crosslink density may regulate fluoride release rate and restrict the extent of the total swelling of the controlled release device.

3.1.1. pH-sensitive Microspheres

We have successively developed a protocol to make a series of pH-sensitive copolymer microspheres by suspension polymerization (Wei, 1995). The hydrophobic monomer in the copolymer microspheres is either styrene (St) or methyl methacrylate (MMA). The pH-sensitive monomer in the copolymer is either N, N-dimethylaminoethyl methacrylate (DMA) or N, N-diethylaminoethyl methacrylate (DEA). Divinylbenzene (DVB) is used as crosslink agent. The swelling behavior of the St/DMA/DVB copolymer microspheres has been well characterized in different buffer systems (Wei, 1995). The swelling onset pH of the copolymer containing 30% DMA (or DEA) remains in the pH

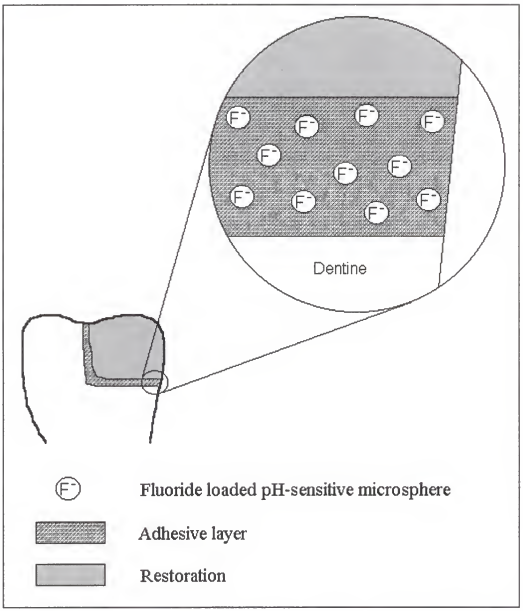


Figure 3.1: Schematical illustration of hybrid pH-sensitive fluoride release systems.

range of 4.5-6.0, close to the "critical pH" of the dissolution of dentine. Furthermore, the pH-sensitive release of a dye (9-aminoacrydine) from the microspheres has also been demonstrated successfully at a pH around 5.5 (Wei, 1995).

The majority of the dental resins and composites are methacrylate based. To ensure good miscibility and adequate bonding between pH-sensitive microspheres and the bonding agent, we decided to use methyl methacrylate (MMA) as the hydrophobic component in the microsphere preparation. To facilitate the preparation of microspheres, less water soluble DEA was used as the pH-sensitive monomer. Preliminary results indicated that the swelling onset pH for MMA/DEA/DVB 69/30/1 microspheres is close to 6 (Figure 3.2) (Yan and Batich, unpublished data). Therefore, the copolymer with a molar ratio of MMA/DEA/DVB 68/30/2 will be used as a baseline throughout this study. The copolymer microspheres with a diameter from 80 to 120 micron will be used as the pH-sensitive core for fluoride release.

3.1.2. Buffer System

It has been found that the hydrophilicity and lipophilicity of the buffer species plays a significant role in pH-sensitive swelling/releasing behavior (Wei, 1995, Wei et al.,1996). Therefore, to precisely characterize swelling/release behavior in vitro, the buffer system should resemble the physiological components as closely as possible. Lactic acid is the primary acid responsible for the active caries (Hojo et al., 1994). In this study, to mimic an active carious attack, we will use a lactic buffer system as the receiving media in all of the pH-sensitive fluoride release studies. The total lactic acid

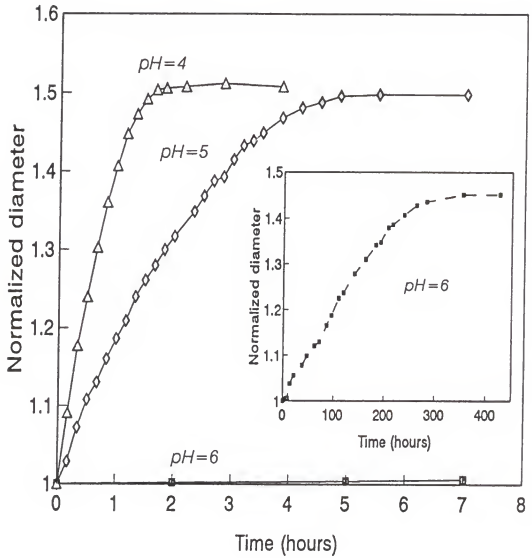


Figure 3.2: pH-sensitive swelling behavior of MMA/DEA/DVB 69/30/1 micorspheres in 0.05 M citric buffers (I=0.3 M) (Yan and Batich, unpublished).

concentration is 0.10 M and the ionic strength of each buffer solution will be maintained at 0.10 M.

3.2. Experimental Plan

3.2.1. Loading of the Fluoride

3.2.1.1 Concept of ion-exchange loading technique

The loading of the fluoride into the MMA/DEA/DVB microspheres remains a primary challenge. Wironen explored several solvent loading techniques to load fluoride (in the form of NaF and KF) into MMA/DEA/DVB 77/20/3 microspheres (Wironen, 1997). With these solvent loading techniques, the NaF loading in the microspheres was below the detectable limit. The highest KF loading in the microspheres was 0.38%wt, which is not high enough for this particular application (Wironen, 1997).

To boost the efficiency of fluoride loading in microspheres, we proposed to use an ion-exchange technique to load the fluoride. The concept is based on the following simple reaction (the aqueous solubility of each reagent is shown in the parentheses):

$$\text{HF (48\%)} + \text{NaCl (37\%)} \longrightarrow \text{NaF (4.2\%)} \downarrow + \text{HCl (37\%)}$$

or

$$\text{KF (94\%)} + \text{NaCl (37\%)} \longrightarrow \text{NaF (4.2\%)} \downarrow + \text{KCl (96\%)}$$

First, we load pH-sensitive microspheres with concentrated hydrofluoric acid (or KF) with traditional solvent loading techniques. Then, the swelled microspheres will be immersed in saturated NaCl solution. In this process, ion-exchange will occur, and the

less soluble NaF will precipitate from the system. The significance of this loading technique is that we can further increase NaF loading levels by repeating the loading circle after the completion of an ion-exchange process.

3.2.1.2 Determine the ideal loading media for solvent loading of fluoride

According to the scheme proposed in the previous section, the first step is to load HF (or KF) into the copolymer successfully. An ideal medium for a solvent drug loading technique should meet the following two criteria: 1)the sample can be swelled extensively in the loading media; and 2) the drug has high solubility in the loading media. The MMA/DEA/DVB 68/30/2 copolymer will swell in acidic environment. Therefore, hydrofluoric acid can be loaded in the copolymer by direct immersion into a concentrated HF solution. However, the pH of the saturated KF aqueous solution is around 10. MMA/DEA/DVB copolymer will not swell in a basic environment. For the loading of KF, organic solvents could be used for swelling the copolymer.

We believe that a certain ratio of acetone/methanol/water solution should be the most efficient for KF loading, since acetone and methanol favor swelling the MMA and DEA sections of the copolymer, while water favors dissolution of KF. To determine the ideal loading media for KF loading, a total of 16 solvent mixtures with various methanol, acetone, and water concentration were investigated for ideal loading efficiency. Each of these solutions were saturated with KF. The copolymer specimens were loaded with KF under the same conditions in these loading media. Then the KF loading efficiency was determined by three different methods: 1) X-ray photoelectron spectroscopy (XPS); 2) ashing the sample below the sublimation temperature of potassium fluoride; 3) releasing

fluoride in acidic environment, then the released fluorine ion was determined by using a fluoride selective electrode.

3.2.2. Release of Fluoride from pH-sensitive Copolymer

We have demonstrated the pH-sensitive release of a dye (9-aminoacrydine) and other therapeutic agents (clotrimazole, chlorhexidine) from St/DMA/DVB and MMA/DEA/DVB copolymer microspheres (Wei, 1995; Wironen, 1997). However, it is not certain that the same copolymer microspheres can be used for the pH-sensitive release of fluorine ions. Not only is the molar mass of fluorine ion very low (atomic weight 19 g/mol), but also fluorine is the element with the highest electronegativity (3.98). Therefore, compared to other halogens, the size of the fluorine ion is abnormally small. In fact, the fluorine ion is the smallest anion we find today. Since the free volume in the polymer could be as high as 10% (even below the glass transition temperature of the polymer), it remains unanswered whether the diffusion of fluorine ions can be restricted by a collapsed copolymer network.

To address this concern, we have prepared MMA/DEA/DVB 68/30/2 copolymer with a bulk polymerization technique. Then the copolymer was loaded with fluoride with the ion exchange technique proposed in the previous sections. Then, fluoride release will be monitored in lactic buffer solution from a pH range from 4.5 to 6.0 at the predetermined time interval. An increasing fluoride release rate is expected in more acidic media. The results of this study will indicate if St/DEA/DVB microspheres can be used as a pH-sensitive gate to release fluoride on demand.

3.2.3. Formulation of Adhesive Layer

To fulfill their therapeutic function, fluorine ions should finally reach the dentine by diffusing through the adhesive layer surrounding the fluoride loaded microspheres. A successful adhesive layer should perform three functions in this specific application:

1) regulate the fluoride release rate

2) restrict excess swelling of the pH-sensitive cores

3) provide an adequate bond between dentin and the dental restoration

In the literature, 3M Scotchbond™ Multi-Purpose Adhesive was frequently referenced due to its adequate bonding strength, as well as its broad acceptance in the dental community. In this study, we used 3M Scotchbond™ MP as the baseline for the formulation of the bonding adhesive. 3M Scotchbond™ MP contains 30-40% hydroxylethyl methacrylate (HEMA) and 70-60 % 2,2-bis [4(2-hydroxy-3-methacryloyloxy-propyloxy)-phenyl]propane (BisGMA). HEMA is a hydrophilic monomer. The equilibrium water uptake of polyHEMA is around 40 wt%. BisGMA is a more hydrophobic monomer. The apparent hydrophilicity and water permeability of the resin mixture can be easily modified by altering the HEMA/BisGMA ratio, and thus, the desired fluoride release rate can be achieved. Also, BisGMA is a four-functional hydrophobic crosslinker. After polymerization of BisGMA, a rigid polymer network was formed. This tightly croslinked polymer network around pH-sensitive microspheres provides adequate mechanical strength of the device. High crosslink density of the adhesive layer around the microspheres could be used to restrict the excess swelling of the microspheres as well.

In this study, a wide range of HEMA/BisGMA ratios from 20/80 to 80/20 was prepared. 0.5% camphorquinone and 0.75% of DMA were added as photosensitive initiator into the resin mixture. Fluoride loaded pH-sensitive microspheres will be mixed with the resin mixture and cured immediately with a dental light curing unit in a Teflon split mould. Then, fluoride release will be monitored in 0.1 M lactic buffer at different pH's from 4.0 to 6.0.

3.2.4. Interface and Shear Bonding Strength Evaluation

The bonding strength between microsphere and adhesive is one of the crucial factors for maintaining overall mechanical properties of the adhesive layer. The microsphere/adhesive interface was observed by using scanning electron microscopy (SEM). Following two methods were used to expose the interface:

1) mechanical bending till the sample breaks;

2) polishing sand paper (up to 1200 grit).

The shear bond strength of all adhesive resin mixtures (with and without microspheres) between human dentine and a composite restorative material will be tested. Extracted human caries-free teeth will be used. Five replicates will be used for each test. The dentin surface will be polished with a series of polishing sand paper (up to 1200 grit) on a water-cooled polishing table. The bonding procedure will follow the procedure according to 3M Scotchbond MP. After bonding, the samples will be kept at 37°C for 48 hr in water. Then bond strength will be tested in shear mode with a tensile testing machine. 3M Scotchbond MP will be used in the control group.

CHAPTER 4
MATERIALS AND METHODS

4.1. Synthesis of pH-sensitive Microspheres

The pH-sensitive microspheres used in these studies were kindly supplied by Dr. William Toreki. These microspheres were prepared by suspension polymerization.

4.1.1. Preparing of the Dispersion Media

The dispersion media used in the suspension polymerization are composed of bentonite (Fisher, lot 914323A), hydroxyethyl cellulose (HEC) (Aldrich, lot 16111CZ) and sodium chloride (Fisher, lot 861673), and distilled water. To facilitate the preparation, hydroxyethyl cellulose was pre-dissolved in DI-water (about 10 % w/v). The actual amount of each component varies slightly according to the change in composition of monomer mixture. Table 4.1 shows a typical recipe of the dispersion media in general.

Table 4.1: Recipe of the Dispersion Media for Suspension Polymerization.

Ingredient	Amount
Bentonite	1.18 g
Hyroxyethyl Cellulose	0.32 g
NaCl	50.6 g
Water	250 ml

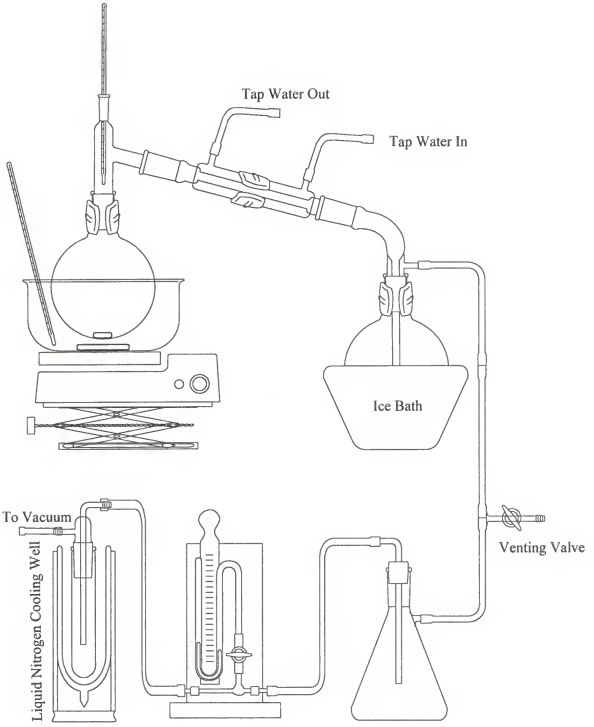


Figure 4.1: Vacuum distillation apparatus.

4.1.2. Preparation of the Monomer Mixture

The monomer mixture contains methyl methacrylate (MMA) monomer (Aldrich, lot 05018TZ), N,N-diethylaminoethyl methacrylate (DEA) (Monomer-Polymer & DAJAC Laboratory Inc., PA, lot 7-18-12), the cross link agent divinylbenzene (DVB) (high purity, Polymer Laboratories, Windham, NH, lot 6-46-1), and the free radical initiator 2,2'-Azo-bis-isobutyronitrile (AIBN) (Aldrich, lot unknown). MMA and DEA monomer were vacuum distilled to remove impurities and polymerization inhibitors. Figure 4.1 shows the distillation apparatus. For MMA, the distillate was collected at 31°C under 52 mmHg vacuum. For DEA, the distillate was collected at 65 °C under 4 mmHg vacuum. DVB was washed with 1 M NaOH (Fisher, lot 7129926) aqueous solution several times in a separatory funnel to remove the inhibitor. AIBN was purified by recrystallization in 50/50 methanol/water solution. All agents were then kept at 2-4 °C before use. The monomer mixture with MMA/DEA/DVB 78/20/2, in molar ratio, along with 0.5% (w/w) AIBN was mixed thoroughly in an Erlenmeyer flask.

4.1.3. Suspension Polymerization

Figure 4.2 shows the apparatus of the suspension polymerization. The agitator (PTFE blade) was stirring at around 350 rpm. About 250 ml of dispersion media and 30 grams of the monomer mixture was used in each reaction batch. The dispersion media was preheated to 65-75 °C while nitrogen was pumped through the reaction apparatus continuously. The temperature was maintained within this range by using a digital temperature controller (Honeywell, DC 2003, Clearwater, FL). When the proper temperature was reached, the monomer mixture was added to the reaction flask through a

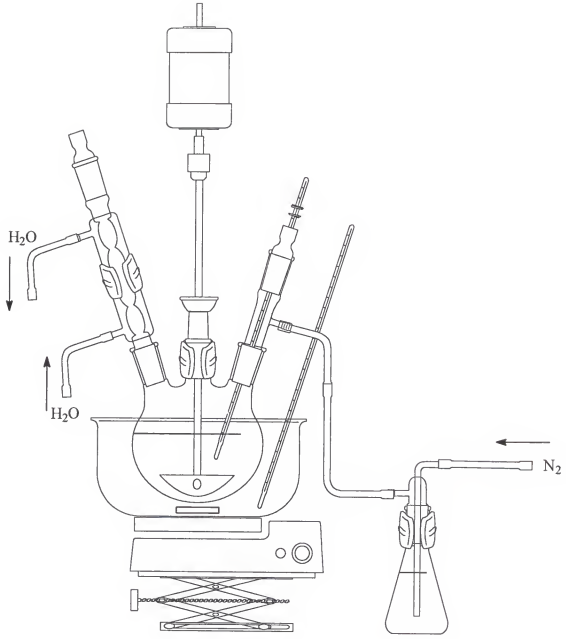


Figure 4.2: Apparatus for suspension polymerization.

funnel. The reaction was allowed to continue at 70°C for about 6 hours, and then the temperature was increased to 85 °C for 1-2 hours to complete the reaction.

4.1.4. Collection of the Microspheres

The entire reaction mixture from the suspension polymerization was transferred into a beaker. Since the density of the copolymer is less than that of the dispersion media, the copolymer microspheres and dross floated to the surface of the dispersion media, while bentonite (which exhibits higher density than the aqueous phase) settled to the bottom of the beaker. After sitting for 2-4 hours, the copolymer microspheres and dross can be collected by using a nylon filter membrane. Then the copolymer microspheres and dross were allowed to settle in a 2 liter capacity graduated cylinder filled with tap water. After settling for several hours, the solid microspheres deposited on the bottom, while the dross, which generally contained air bubbles, floated on the surface. After removing the dross, the copolymer microspheres were washed several times with tap water and DI water. Then, spheres within a defined size range were isolated by pouring an aqueous suspension of them through a series of sieves. To remove any possible monomer residue entrapped inside, the selected microspheres were allowed to fully swell in 50/50 acetone/water solution. Finally, the microspheres were dried in a vacuum oven at 60-70°C for 24-48 hours.

4.2. Preparing of MMA/DEA/DVB 68/30/2 Copolymer Film

The MMA/DEA/DVB copolymer film used in these studies was prepared by bulk polymerization. Polymerization was performed between two glass plates which were

separated with a silicone tube (OD ≈1.5 mm). To facilitate removing of the copolymer film from the glass plates after the completion of the polymerization, the surface of the glass plates was sprayed with Tri-Epoxy Mold Release Spray (Tri-Dynamics Dental Co., Inc., NJ), and then dried in an oven at 70°C for 3-4 hours. Figure 4.3 shows the apparatus for making the copolymer films using bulk polymerization. The monomer mixture was prepared similarly as mentioned previously in section 4.1.2. The molar ratio of MMA/DEA/DVB in the monomer mixture was 68/30/2. The monomer mixture was then injected into the reaction apparatus through a needle. The whole set-up was incubated in an oven at 70-75°C for about 16 hours. Then the copolymer film was cut with a water cooled low speed diamond wheel saw (South Bay Technology Inc., CA) to approximately 5x8x1 mm^3 in size. All the specimens were ultrasonicated in distilled water for 30 minutes, and dried in an oven at 60-70 °C for about 24 hours. The specimens were kept in a desiccator before use.

4.3. Preparing of Lactic Buffer Solution

Lactic buffer solutions were prepared by mixing calculated amounts of lactic acid (85%) (Fisher, lot 970874) and sodium hydroxide (Fisher, lot 975744) in distilled water. The pH of the buffer solutions was determined with a pH meter (EA920, Orion Research, Boston, MA.). In some cases, hydrochloric acid (HCl) (37%) (Mallinckrodt, St Louis, MO.) was used for fine adjustment of the pH of the buffers. The total lactic acid concentration was maintained at 0.10 M for all the buffer solutions prepared. The ionic strength of the buffer solutions was also maintained at 0.10 M by addition of computed

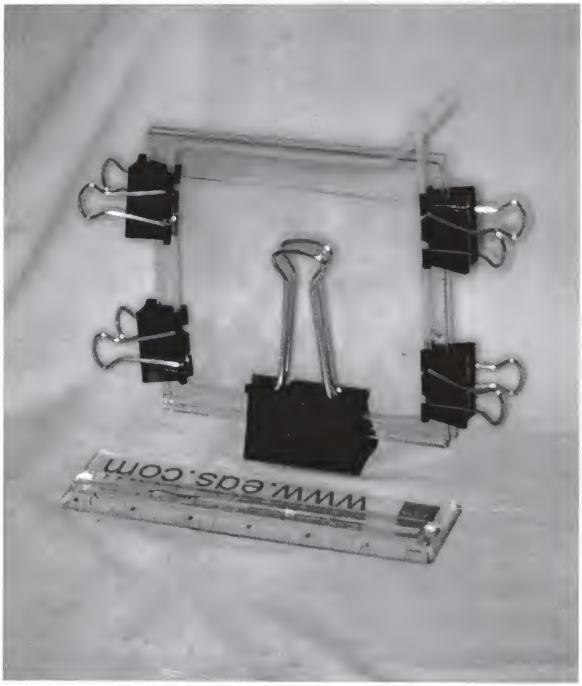


Figure 4.3: Apparatus of bulk polymerization for preparation of the copolymer films.

amounts of sodium chloride (NaCl) (Fisher, lot 974188). Thymol (Fisher, lot 917385) was added for preservative proposes. Table 4.2 shows the recipes of these buffer solutions. Unless otherwise noted, these lactic buffer solutions were used throughout the following studies.

Table 4.2: Recipes for preparing 0.1 M lactic buffer solution (I = 0.1 M).

pH	Lactic Acid (85%)	NaOH	NaCl	H_2O	Note
3.5	42.35 (g)	4.81 (g)	16.29 (g)	4000 (ml)	Adjusted w/ HCl
4.0	42.35 (g)	9.26 (g)	9.83(g)	4000 (ml)	
4.5	42.35 (g)	13.01 (g)	4.36 (g)	4000 (ml)	
5.0	42.35 (g)	14.92 (g)	1.58 (g)	4000 (ml)	
5.5	42.35 (g)	15.64 (g)	0.49 (g)	4000 (ml)	
6.0	42.35 (g)	15.88 (g)	0.16 (g)	4000 (ml)	Unstable
6.5	42.35 (g)	15.96 (g)	0.05 (g)	4000 (ml)	Very Unstable

4.4. Loading of Fluoride

To facilitate the sample handling, the MMA/DEA/DVB 68/30/2 copolymer films prepared as previously described in section 4.2 were used exclusively throughout the following fluoride loading experiments. To minimize possible interactions between the

silicate glass and fluorine ions, all containers, stir bars, and pipet tips, exposed to the fluorine ions, are made of plastic throughout this study, unless otherwise noted.

4.4.1. Na-H Ion-exchange Technique

MMA/DEA/DVB copolymer is capable of swelling in the acidic media. The two-step scheme of the Na-H exchange fluoride loading procedure is: 1) load the copolymer with concentrated hydrofluoric acid (HF) in an acidic environment, 2) replace proton ions (H^+) with sodium ions (Na^+) by ion-exchange.

The copolymer films were immersed in concentrated hydrofluoric acid solution (48-50%) (Fisher, lot 925386) at room temperature for 24-48 hours. Then, they were retrieved with plastic tweezers. After removing the excess acid on the surface of the specimen by blotting with a lab tissue, the specimens were immersed in an excess NaCl (Fisher, lot 974188) saturated aqueous solution at room temperature for 24-48 hours. The specimens were blotted with lab tissues to remove any excess solution on the surface. Finally, the specimens were retrieved and allowed dried in the air at room temperature for about 6-8 hours, and then dried in a vacuum oven at 70-85°C for another 12 hours. The fluoride loading level was assessed as described later in this text.

4.4.2. Na-K Ion-exchange Technique

The two-step scheme of Na-K exchange fluoride loading technique is: 1) loading highly water soluble potassium fluoride (KF) into the MMA/DEA/DVB copolymer, 2) replacing the potassium ions (K^+) with Na^+ ions by ion-exchange. To load KF in the copolymer, organic solvents (methanol and acetone) will be employed to swell the copolymer.

4.4.2.1. Optimum loading medium

To determine the optimum loading media for KF, a total of 16 mixture solutions of methanol (Fisher, lot 963102), acetone (Fisher, lot971210), and water were prepared in 4 groups, as indicated in Table 4.3. For each group the methanol/acetone ratio (V/V) is fixed. The water content in each group varies in the range from 0-15 %vol. Then each solution was saturated with potassium fluoride (Acros, lot B0029624B) by addition of an excess amount of KF.

MMA/DEA/DVB 68/30/2 copolymer slabs were weighed precisely (±0.01 mg) on an electronic analytical balance (Mettler HL 52, Mettler Instrument Corp. NJ). Then, five of the copolymer slabs were immersed in each solution at room temperature for 12-24 hours. After the specimens were fully swelled, they were retrieved from the solution. The excess solution on the surface of the specimens was removed by blotting with a lab tissue. Then, for Na-K ion-exchange, the specimens were immersed in an aqueous solution, saturated with both NaCl (Fisher, lot 974188) and NaF (Fisher, lot 975627), at room temperature for 24-48 hours. The agitation of the Na-K ion-exchange solution was provided by an electronic rotator (Fisher, model 346). All specimens were retrieved at the same time and dried under the exact same condition as described in section 4.4.1. Then, fluoride loading level was determined as described later in section 4.5. The highest fluoride loading level in the copolymer slabs indicated the optimum loading media.

4.4.2.2. Influence of time of Na-K ion-exchange

The influence of the time of K-Na ion exchange on the fluoride loading efficiency was also investigated in a similar way as described in the previous section. All

Table 4.3: Solutions for determination of optimum loading medium for KF.

Water Content	Methanol/Acetone Ratio (V/V)			
	85/15	70/30	55/45	40/60
0 %vol	X	X	X	X
5 %vol	X	X	X	X
10 %vol	X	X	X	X
15 %vol	X	X	X	X

copolymer slabs were immersed in the KF saturated methanol/acetone 55/45 (V/V) mixture solution for about 24 hours. They were all retrieved from the KF loading solution at the same time. Then the copolymer specimens were transferred to Na-K ion-exchange solution. At each predetermined time, five specimens were retrieved from the Na-K ion-exchange solution. After drying, the fluoride loading level was assessed as described later in section 4.5.3.

4.5. Determination of Fluoride Loading Level

4.5.1. X-ray Photoelectron Spectroscopy (XPS)

The XPS analysis was performed in the Major Analytical Instrumentation Center at the University of Florida. The surface of the specimen was scraped with a surgical blade to expose the fresh surface of the specimen. The blank MMA/DEA/DVB copolymer is composed of four elements: carbon, hydrogen, oxygen, and nitrogen. After

fluoride loading, the specimen is composed of six elements theoretically: carbon, hydrogen, oxygen, nitrogen, sodium, and fluorine. Because of possible incompletion of the Na-K ion-exchange process, a certain amount of potassium is also expected to exist in the fluoride loaded specimen. The mount of potassium detected is assumed to indicate the efficiency of the Na-K ion-exchange process. A Kratos model XSAM-800 X-ray photoelectron spectroscopy (Manchester, England) was used for the analysis. C_{1S}, O_{1S}, N_{1S}, F_{1S}, Cl_{2P}, K_{2P}, and Na_{1S} peaks were detected at 285 eV, 536 eV, 399 eV, 693 eV,197 eV, 299eV, and 1075 eV, respectively. The X-ray gun was operated at 12 kV and 9 mA, and the analysis chamber pressure was maintained at 10^{-7}-10^{-8} torr during the analysis. Quantification of the spectra was performed using DS800 Kratos software on a Digital computer system.

4.5.2. Ashing

The porcelain crucibles used in this experimental were pre-fired in an furnace (Rapid Temp Furnace, C-M Inc., NJ) at 900°C for 24 hours, and then kept in a desiccator until use. Each crucible and the fluoride loaded copolymer specimen were weighed individually in an electronic analytical balance (±0.01 mg). The specimen was placed in a crucible individually and heated in the furnace at 550 ± 20 °C for 48 hours. Then the crucibles were retrieved from the furnace and kept in a desiccator for cooling. After reaching room temperature, the crucibles were weighted individually again on the same balance. The increase in weight of the crucibles (compared with weight of the initial empty crucibles) indicates the amount of fluoride loaded in the specimen. The fluoride

loading level (Fluoride %wt) was determined by equation 4.1. Three replicates were used for each loading condition.

$$MF\% = \frac{\text{Weight of loaded fluoride}}{\text{Weight of fluoride loaded copolymer}} x100\% \qquad \text{(Eqn. 4.1)}$$

4.5.3. Fluoride Selective Electrode

4.5.3.1. Working curve

Fluoride loaded specimens were weighted accurately in an analytical balance. The loaded fluoride was allowed to release completely by immersing the specimen individually in 20.0 ml 0.1 M lactic buffer at pH 3.5 for 4-5 days. Then 1.0 ml of the eluent was pipetted in a plastic vial for analysis. An Ion Analyzer EA 920 (Orion Research, Boston, MA.) equipped with a fluoride selective electrode (Fisher, Accumet 13-620-529) was used in this measurement. The fluoride standard (100 ppm F^-) (Orion Research, lot. S01) was used as supplied. A set of standard fluoride solutions with fluorine ion concentration of 1.0, 2.0, 5.0, 10.0, 20.0 ppm was prepared by dilution of the 100 ppm standard with DI water in plastic volumetric flasks. Total ionic strength adjustment buffer (TISAB) (Orion Research, lot ZS1) was used as supplied. TISAB to sample ratio (V/V) was maintained to a 1:1 ratio for all measurements. The fluoride selective electrode was calibrated with the standard solutions. An electric potential (mV) was read as the direct result of the measurement. A linear working curve can be obtained by plotting mV versus $log[F^-]$. A typical working curve was shown in Figure 4.4. The

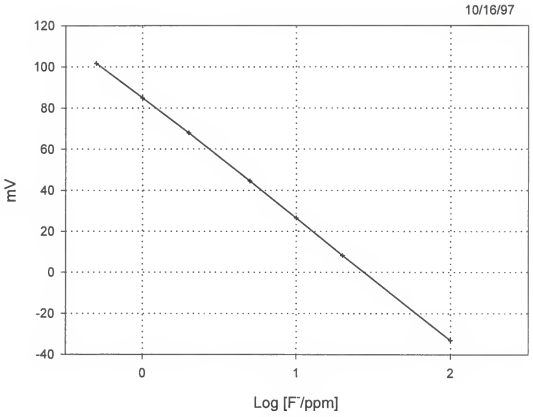


Figure 4.4: A typical working curve for fluoride selective electrode.

linear regression coefficient of the working curve is normally higher than 0.98 in the range from 0-100 ppm.

4.5.3.2. Measurement

Each fluoride loaded copolymer slab was weighted individually on an analytical balance (±0.1 mg). About 10.0 mg of the specimen were immersed in 20.0 ml 0.1 M lactic buffer of pH 3.5 for 3 days at room temperature in a plastic container. The solution was well agitated to assure a homogeneous concentration, and the elute was mixed with the same volume of TISAB in a plastic vial. Then the fluorine ion concentration was measured with the fluoride electrode. The fluorine ion concentration was obtained from the working curve which was established on the same day as the measurement. The fluoride loading level (F^-%wt) was computed with equation 4.2.

$$F^-\% = \frac{\text{Weight of loaded fluorine ion}}{\text{Weight of fluoride loaded copolymer}} x100\% \qquad \text{(Eqn. 4.2)}$$

4.6. Fluoride Release through a pH-sensitive Copolymer

4.6.1. Loading of the Fluoride in a pH-sensitive Copolymer

The Na-K ion-exchange fluoride loading technique, as described previously in section 4.4.2, was used for fluoride loading in a pH-sensitive copolymer for this study. KF saturated acetone/methanol 45/55 (V/V) solution was used as the KF loading solution. About 100 MMA/DEA/DVB 68/20/2 copolymer slabs were immersed in KF loading solution in a plastic centrifuge tube at room temperature for 24-36 hours under constant

agitation. All specimens were retrieved from the KF loading solution. Then they were divided into two groups. The specimens in Group A were allowed to air-dry at room temperature for 24 hours directly. The specimens in Group B were transferred to a Na^+ rich solution for Na-K ion-exchange as described previously in section 4.4.2. Then all the specimens were dried in a vacuum oven at 70-85°C for over 12 hours. All specimens were kept in a desiccator before use.

4.6.2. pH-sensitive Release of the Fluoride in Lactic Buffers

Fluoride release behavior from the specimens in Group A and B were monitored by the fluoride selective electrode in 0.1 M lactic buffers in the pH range from 4.0 to 6.0. Each fluoride loaded copolymer slab was weighed individually on an analytical balance (±0.01 mg). About 10.0 mg of the specimen was placed in a plastic vial containing 20.0 ml 0.1 M lactic buffer solution at a given pH. At the predetermined time, the specimen was retrieved from the solution. After blotting the surface with a lab tissue, the specimen was transferred to another vial containing 20.0 ml buffer with the same pH. Then the fluorine ion concentration in the elutate was measured with a fluoride selective electrode as described in section 4.5.3. For comparison, the cumulative fluorine ion release was normalized to 50 mg of fluoride loaded specimen. According to the Equation 4.2, the fluoride loading level in the specimen was calculated based on data at the completion of the release at low pH.

4.7. Fluoride Release through a Bonding Adhesive Layer

4.7.1. Preparing of Bonding Adhesive

BisGMA (ESSCHEM Inc., lot 419-19-03), and camphorquinone (Aldrich, lot DW 06724AW) were used as received. HEMA (Aldrich, lot 15509LQ) was vacuum distilled at 61°C under 4 mmHg before use. N,N-dimethylaminoethyl methacrylate (DMA) (Aldrich, lot 05030LY) was vacuum distilled at 75°C under 8 mmHg before use. The bonding adhesives were prepared by hand mixing BisGMA and HEMA at the desired ratio (Wt/Wt). Camphorquinone and DMA were added to the resin mixture as the photo-sensitive polymerization initiator. Then each bonding adhesive was kept in a black plastic container before use. Table 4.4 shows the recipes for preparing these bonding adhesives.

Table 4.4: Recipes for preparing BisGMA/HEMA bonding adhesives.

Adhesive Composition (Wt/Wt)	BisGMA (g)	HEMA (g)	DMA (g)	Camphorquinone (g)
BisGMA/HEMA 20/80	20.40	81.60	0.691	0.355
BisGMA/HEMA 35/65	35.22	65.41	0.715	0.357
BisGMA/HEMA 50/50	50.03	50.16	0.695	0.344
BisGMA/HEMA 65/35	65.18	35.11	0.706	0.363
BisGMA/HEMA 80/20	80.21	20.35	0.722	0.360

4.7.2. Loading of Fluoride in pH-sensitive Microspheres

MMA/DEA/DVB 78/20/2 microspheres prepared in section 4.1 were used in this test. Fluoride was loaded into the copolymer microspheres in the very similar way as described in section 4.6.1. The microspheres in group A were allowed to air-dry at room temperature after being retrieved from the fluoride loading solution. The microspheres in group B went through the Na-K ion-exchange process as described previously. The excess solution on the surface of the microspheres was removed by vacuum filtration with a nylon membrane (Magna, lot 78142). The opening size of the membrane is 10.0 micron. The microspheres were dried under the same conditions as described in section 4.6.1. Then the microspheres were once again rinsed with NaOH solution (pH 8-9) to remove any possible fluoride crystals formed on the surface of the microspheres during the drying. The microspheres were finally dried at 70-85°C under vacuum for about 24 hours. They were then kept in a desiccator before use.

4.7.3. Preparing of Specimens

The specimen was prepared in a PTFE split mould (Figure 4.5). The cavity of the mould is 8.9x7.0x1.3 mm^3. The fluoride loaded microspheres were mixed with bonding adhesive resins mechanically. The microspheres to resin ratio was maintained at 1:2 (Wt/Wt) for all of the specimens. The resin mixture was poured into the split mould immediately after mixing. After covering with a microscopic cover glass (Fisher, PA), the resin mixture was cured with a visible light curing unit (Visilux 2^{tm}, 3M Dental products, MN) for 2 minutes. The specimens were then kept in a desiccator before further tests.

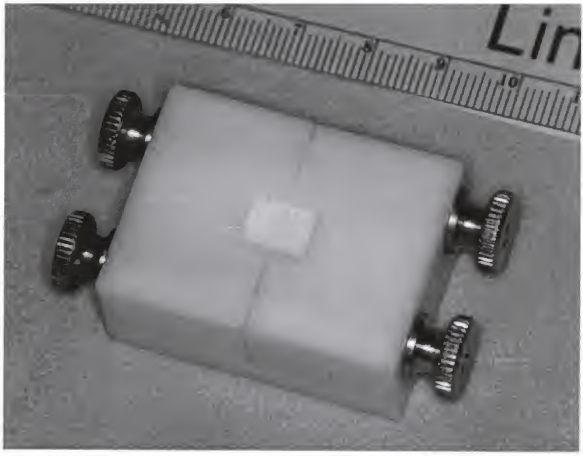


Figure 4.5: PTFE split mould with a rectangular cavity of 7.0 x 8.9 x 1.3 mm^3.

4.7.4. pH-sensitive Release of the Fluoride in Lactic Buffers

The fluoride release from the specimens was monitored by a fluoride selective electrode in 0.1 M lactic buffers in the pH range from 4.0-6.0 in exactly the same fashion as described in section 4.6.2.

4.7.5. Investigation of Microsphere/Adhesive Interface and Fluoride Release Through the Adhesive Layer

A JEOL JSM 35 scanning electron microscope (SEM) was utilized for the observation of the interface between microsphere and bonding adhesive layer. Two methods were used to expose the microsphere/adhesive interface: 1)bend the specimen to break; 2)polish the specimen up to 1200 grit sand paper on a water cooled polishing table. Then the specimens were fixed on an aluminum sample substrate by using a double side tape (Scotch™). The specimens were sputter coated with Au-Pd for approximately 6 minutes. To prevent overheating of the specimen under the electron beam, the acceleration voltage used was 5 Kv.

4.8 Shear Bonding Strength between Dentin and Restoration

4.8.1. Preparing of Dentin Surface

Extracted human molars were used in this experiment. To expose the sound dentin, the enamel on the occlusal surface of the teeth was removed by cutting laterally with a water cooled low speed diamond wheel saw. Then the tooth was embedded individually in a dental dye stone (Labstone®, Kulzer Dental Product, lot unknown) in a plastic cylindrical mould and allowed to set for about 8 hours. The dentin surface of the

teeth was polished on a water cooled polishing table with a series of sand papers (from 400-1200 grit). The polished dentin surface was perpendicular to the axis of the cylinder of the specimen. The tooth was stored in water before further testing.

4.8.2. Bonding between Dentin and Restoration

A dental composite restorative material (Z100, 3M Dental Products, lot 5904A3) was bonded to the polished human dentin via dental bonding adhesive prepared as described in section 4.6.1 and 4.6.3. The phosphoric etchant and primer in the Scotchbond MP system (3M Dental Products, St. Paul, MN) were used for all specimens. The adhesive in Scotchbond MP^{tm} system was used as a control. The bonding procedure was performed as suggested by the manufacturer. The dentin surface was etched with the phosphoric etchant for 15 seconds, then rinsed with water for 20 seconds. After blotting dry the dentin surface, the primer was painted on the surface, and then blown with compressed air for 5 seconds. The experimental bonding adhesive was painted on the surface and light-cured for 30 seconds immediately. A split PTFE mould with a cavity of 3.0 mm in diameter (Figure 4.6) was placed at the desired place on the dentin surface. The cavity of the mould was packed with the restorative resin in two increments and each increment was light-cured for 45 seconds. After bonding, the specimens were immersed in water and stored in an incubator at 37°C for 48 hours before further testing.

4.8.3. Shear Bonding Strength Test

After the incubation, the shear bonding strength of each specimen was tested. The specimen was placed in an universal tensile testing machine (Model 1125, Instron) equipped with a 50 kg tension load cell. A steel wire (with 1x1 mm^2 square cross-

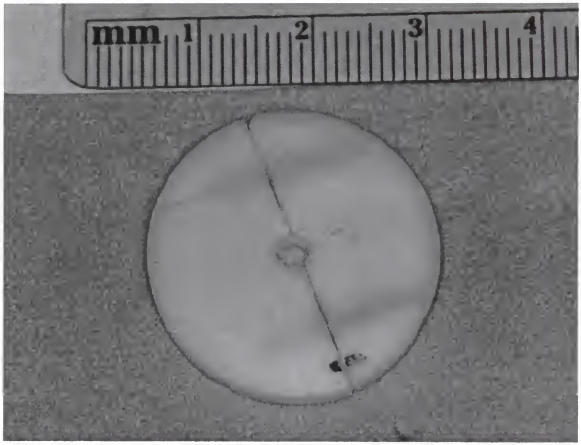


Figure 4.6: PTFE split mould with a circular cavity of 3.0 mm in diameter.

section) loop surrounded the base of the composite knob (Figure 4.7 and 4.8). The wire was pulled in tension till failure occurred at the dentin/composite interface. The cross-head speed was maintained at 0.5 mm/min for all specimens. The force at the failure and the type of the debonding were recorded. The shear bonding stress was obtained based on the equation 4.3.

$$\sigma_{\max} = \frac{4 \times F_{\max} \times g}{\pi \times d^2} \qquad \text{(Eqn 4.3)}$$

where, σ_{max} is bonding strength (in MPa), F_{max} is force at failure (in Kg), g is the acceleration of gravity, d is the diameter of the bonded area (in mm).

Figure 4.7: Shear bond test apparatus.

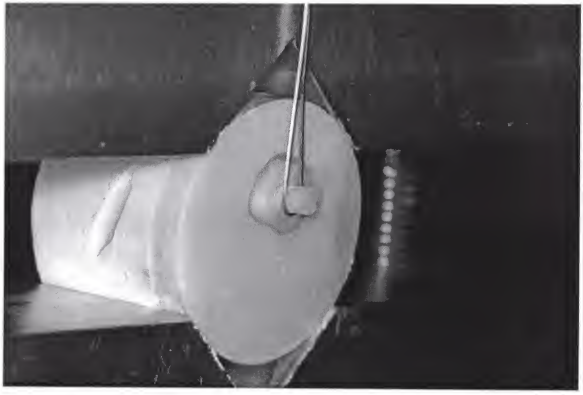


Figure 4.8: Specimen holder in shear bond test apparatus.

CHAPTER 5
RESULTS AND DISCUSSION

5.1. Loading of Fluoride

5.1.1. Difficulties and Solutions

Successful loading of the fluoride in the pH-sensitive copolymer is one of the most important steps for this project. A solution loading technique is commonly selected for drug loading of a polymeric matrix, because of its simplicity, versatilily, and low cost. Solution loading techniques include two steps: 1) swelling the specimen fully in a solvent containing a high concentration of the drug; 2) evaporating the solvent from the specimen. However, loading an ionic inorganic drug into an organic matrix by solvent loading technique is challenging. Because sodium fluoride has a low solubility in common solvents (4.2% in water, at 25°C), it is impossible to obtain a meaningful NaF loading in the copolymer by the solvent loading technique directly. Although potassium fluoride exhibits a much high solubility in water and methanol (97.2% in water at 25°C, 13-15% in methanol at 25°C), the direct solvent loading technique yields extremely low loading levels (about 0.005%KF) (Wironen, 1997). The major problem is that the inorganic species exhibit a poor miscibility in the organic matrix. Therefore, as the solvent evaporates during the loading process, the inorganic species tends to salt-out from the organic matrix. To solve this problem, Wironen proposed using a freeze-drying technique to remove solvent from the specimen, and then the collapsed state of the

copolymer structure was obtained by annealing between the glass-transition temperature and the melting point of the copolymer (1997). The Freeze-drying loading technique is better, but yields only 0.38% of KF loading.

As proposed in section 3.2.1, an ion-exchange technique provides a promising approach for loading sodium fluoride into the copolymers. The principle of this technique is to precipitate less soluble NaF inside the swollen copolymer network. Theoretically, the NaF loading level could be increased by repeating the loading cycles, since the NaF loaded in the preceding cycle would not dissolve, as long as the loading media remains in saturation.

5.1.2. Na-H Ion-exchange Fluoride Loading Technique

The MMA/DEA/DVB 68/30/2 copolymer slabs swelled extensively after immersing in 48% hydrofluoric acid solution for about 24 hours. As they were transferred to a beaker containing NaCl saturated solution for the Na-H ion exchange, white crystals precipitated immediately on the surface of the copolymer slabs. By the end of ion-exchange process, the specimen was completely covered with the white crystals and the size of specimen reduced close to the original size. The white precipitate could be easily removed with a lab tissue. The copolymer specimens remained as completely transparent as the original. Subsequent fluoride loading level analysis (with a fluoride electrode) confirmed that the fluoride loading level in the copolymer specimen approached zero.

The white precipitate accumulated on the surface of the specimen was believed to be sodium fluoride. This observation confirmed that the sodium-proton ion exchange did

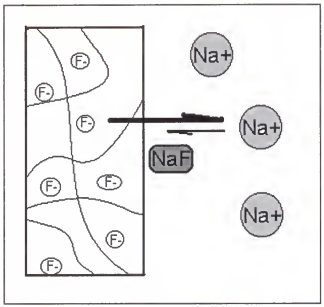
occur when the specimen was immersed in NaCl saturated solution. Unfortunately, NaF precipitated on the surface of of the specimen rather than inside the polymer network. This is because fluorine ion (F^-) is smaller than sodium ion (Na^+). During the ion-exchange, F^- moved more freely in the copolymer network than its counter ion, the more bulky Na^+. Therefore, during the ion-exchange process, fluorine ions diffuse much faster outwards from the polymer network than sodium ions diffuse inwards (Figure 5.1).

If the difference in mobility of fluorine and sodium ions is the cause for NaF precipitating on the surface of the specimen, theoretically, NaF could still be loaded inside the specimen by reversing the loading procedure, that is, load NaCl into the copolymer first, then perform the ion-exchange in concentrated HF solution. Unfortunately, we were not successful in loading NaCl in the copolymer used in this project. MMA/DEA/DVB copolymer swells in either acidic environment or in organic solvent. In an acidic environment, due to the protonation of the amine group of DMA, the copolymer chain carries positive charges which repels the sodium ion (Na^+) with the same charge. Also, we are not successful in finding an organic solvent to dissolve significant amounts of NaCl. However, this technique may be valuable for loading fluoride in another type of copolymer, such as a copolymer based on methacrylic acid (MAA) (which carries negative charges in a basic environment).

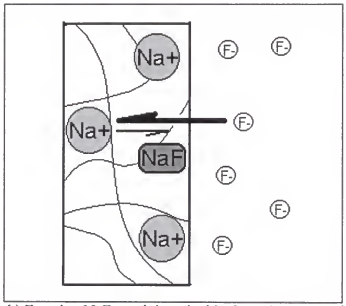
5.1.3. Na-K Ion-exchange Fluoride Loading Technique

5.1.3.1. Preliminary test for KF loading

The MMA/DEA/DVB 68/30/2 copolymer slabs swell remarkably after immersing in a methanol/acetone 50/50 solution saturated with KF. The copolymer slabs remained



a) Forming NaF precipitate outside the polymer matrix
by loading fluorine ion in the polymer matrix first.



b) Forming NaF precipitate inside the polymer matrix
by loading sodium ion in the polymer matrix first.

Figure 5.1: Mobility of fluorine ion and sodium ion on formation of NaF.

transparent in the KF loading medium. A portion of the copolymer specimens were retrieved from the KF loading medium, and allow to dry in the air for 24 hours. After drying, the specimens turned translucent with time. No crystals could observed on the surface of the specimen. After drying in a vacuum oven, the translucency of the specimen reduced greatly. If stored in the air for about 8 hours, the specimen turned translucent again. This observation may correlate with the formation of potassium fluoride hydrate by absorbing moisture from air. In a succeeding flame analysis, a light purple blue flame was observed, which indicated the existence of potassium in the specimen. These observations qualitatively confirmed that there was potassium fluoride inside the copolymer available for the ion-exchange.

5.1.3.2. Medium for KF loading

The loading level depends on both the extent of swelling of the copolymer and the KF concentration in the loading medium. As detailed in section 3.2.1.2, a series of solutions containing methanol, acetone, and water was investigated as possible loading media. The solubility of KF in these solution was determined as shown in Table 5.1. Potassium fluoride was loaded in the MMA/DEA/DVB 68/30/2 copolymer with the solution loading technique directly. Table 5.1 shows the KF loading level in the corresponding solutions determined by ashing at 550°C.

As we expected, the solubility of KF in the mixture solutions increased with the increasing water content in each group with fixed methanol/acetone ratio. Also, with the same water content, the solubility of KF decreased with the increasing of acetone content. However, as shown in Table 5.2, the KF loading in the copolymer was not consistent

Table 5.1: Solubility of potassium in methanol/acetone/water mixture solution (g/100ml).

	Methanol/Acetone (V/V)			
	85/15	70/30	55/45	40/60
0%Vol H_2O	6.3 ± 0.3	4.7 ± 0.3	3.9 ± 0.3	2.0 ± 0.0
5%Vol H_2O	9.5 ± 0.2 (a)	8.1 ± 0.1 (a)	5.5 ± 0.4 (a)	Unstable (c)
10%Vol H_2O	12.8 ± 0.2 (a)	9.2 ± 0.1 (a)	Unstable (b)	Unstable (c)
15%Vol H_2O	15.9 ± 0.8 (a)	13.1 ± 0.6 (a)	Unstable (b)	Unstable (c)

a. Forming potassium hydrate precipitation after 4 hours.
b. Showing phase separation after 15 minutes due to portion of water salting out.
c. Forming potassium hydrate crystal immediately.

Table 5.2: KF loading level in MMA/DEA/DVB 68/30/2 copolymer from KF saturated methanol/acetone/water mixture solution (determined by ashing technique).

	Methanol/Acetone (V/V)			
	85/15	70/30	55/45	40/60
0%Vol H_2O	2.29	1.69	1.75	2.45
5%Vol H_2O	1.53	1.03	1.23	NA
10%Vol H_2O	1.15	1.00	NA	NA
15%Vol H_2O	0.69	0.87	NA	NA

with the solubility of KF in the mixture solution. In each group with a fixed methanol/acetone ratio, the KF loading level decreased with the increasing water content of the loading solution. This result could be correlated to the extreme hydrophilic nature of KF. In fact, as shown in Table 5.1, all of the mixture solutions containing water were relatively unstable. Within several hours after the preparation, KF hydrate precipitated from those mixture solutions. In some cases, phase separation/precipitation occurred shortly after the preparation of the solution. As a result, the potassium fluoride concentration which was actually available for loading may have been reduced. In addition, the KF hydrate precipitation could form inside of copolymer network. Therefore, the formation of the bulky KF hydrate in the copolymer network may also contribute to the reduced KF loading level. For this reason, all the mixture solutions containing water were eliminated as candidates for KF loading media for further tests.

Observing the influence of methanol/acetone ratio on the KF loading in Table 5.2, we found that the highest loading level was obtained in the methanol/acetone 40/60 solution, in spite of the lowest solubility of KF being found for that solution (Table 5.1). The high KF loading in methanol/acetone 40/60 solution was attributed to the extensive swelling of the copolymer. In some cases, the swelling force was even higher than the tensile strength of the copolymer network, which caused cracks in the specimens. Obviously, methanol/acetone 40/60 solution can not be the ideal loading media for this study.

The second highest KF loading was found in methanol/acetone 85/15 solution (Table 5.2), due to the relatively high KF solubility. However, it was not selected as the KF loading medium because the swelling extent of the copolymer was relatively low in

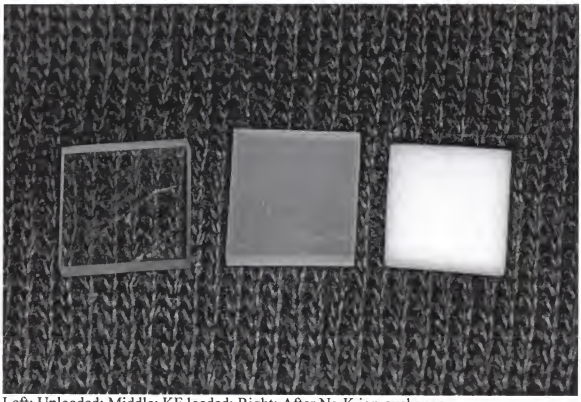
this solution. This low swelling extent of the copolymer made succeeding Na-K ion-exchange less efficient. Considering all of the factors above, methanol/acetone 55/45 solution was used as the KF loading medium throughout the following tests, unless otherwise noted.

5.1.3.3. Medium for Na-K ion-exchange

We used an aqueous solution saturated with both NaCl and NaF as the Na-K ion-exchange medium. The saturated NaCl aqueous solution was used as the Na-K ion-exchange medium. In addition, the ion-exchange medium was also saturated with NaF to prevent dissolution of newly formed NaF inside the copolymer network. As KF loaded specimens transferred into Na-K ion-exchange medium, the specimens turned translucent within 2 hours. There were no obvious crystals forming on the surface of the copolymer. This was the first direct qualitative evidence showing that Na-K ion-exchange occurred in the copolymer network. In a succeeding flame analysis, a bright yellow flame was observed, which indicated the existence of sodium in the specimen. In the following studies, we used an aqueous solution which was saturated with both NaCl and NaF as the Na-K ion-exchange medium throughout the following tests in this dissertation. Figure 5.2 shows the visual comparison of fluoride loaded copolymer specimens.

5.1.3.4. Influence of Na-K ion-exchange time on fluoride loading level

To investigate the influence of Na-K ion-exchange time on the fluoride loading level in the copolymer, forty MMA/DEA/DVB 68/30/2 copolymer slabs were loaded with KF in KF saturated methanol/acetone 55/45 solution in same batch. Then all the specimens were transferred in a beaker containing K-Na ion-exchange medium



Left: Unloaded; Middle: KF loaded; Right: After Na-K ion-exchange

Figure 5.2: Visual comparison of fluoride loaded copolymer specimens.

simultaneously. At the predetermined time, five specimen were retrieved from the beaker. After drying, the fluoride loading level was determined with a fluoride selective electrode as described in section 4.5.3. Figure 5.3 shows the influence of Na-K ion-exchange time on the fluoride loading level in the copolymer. Figure 5.3 shows that a rapid increase in fluoride loading level occurred within the first several hours after Na-K ion-exchange started. The Na-K ion-exchange was approaching equilibrium after 48 hours. This result indicates that the fluoride loading level in the copolymer could be controlled by altering the duration of Na-K ion-exchange.

5.1.3.5. Influence of KF concentration in loading medium on fluoride loading level

To control the fluoride loading level, the influence of KF concentration in KF loading medium on the fluoride loading level in the copolymer was also investigated. In this test, the KF loading media contained 55/45 methanol/acetone (V/V) solution, as before. However, the KF concentrations in the loading media were controlled at 40%, 50%, 60%, 70%, 80%, and 90% of the KF saturation concentration, respectively. Five MMA/DEA/DVB 68/30/2 copolymer slabs were immersed in each loading medium for 24 hours. Then they were retrieved and transferred to a beaker containing K-Na ion-exchange medium simultaneously. All specimens were kept in K-Na ion exchange medium for 48 hours. Then, after drying, the fluoride loading level was determined with a fluoride selective electrode as described in section 4.5.3. Figure 5.4. shows the dependency of fluoride loading on the KF concentration in KF loading media. This result indicates that controlling the KF concentration in the loading media is a more efficient way to control the fluoride loading level in the copolymer.

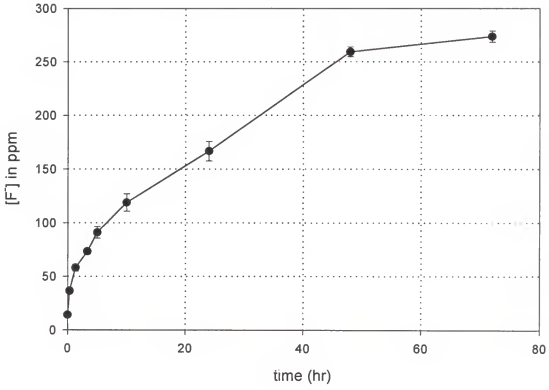


Figure 5.3: Influence of Na-K ion-exchange time on the fluoride loading level in the MMA/DEA/DVB 68/30/2 copolymer (normalized to 0.05g copolymer sample in 10.0 ml lactic buffer).

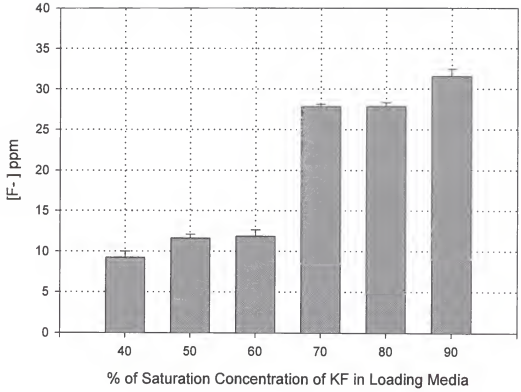


Figure 5.4: Influence of KF concentration in KF loading media on the fluoride loading level in the MMA/DEA/DVB 68/30/2 copolymer.

5.2. Determining of Fluoride Loading Level

5.2.1. X-ray Photoelectron Spectroscopy (XPS)

X-ray Photoelectron Spectroscopy (XPS), also referred to as electron spectroscopy for chemical analysis (ESCA), is a versatile surface analysis technique. XPS provides the atomic chemical composition both quantitatively and qualitatively within the 50 Å below the surface of the specimen.

Based on the photoelectric effect, XPS used Mg Kα radiation to bombard the surface of the specimen, which caused photo-electron emission. The ejection of core electrons emits discrete energy values which relate to the binding energy and the exciting radiation energy of the electron as shown in the equation 5.1.

$$E_b = h\upsilon - E_k - \Phi \qquad \text{(Eqn. 5.1)}$$

where, E_b is the bonding energy, h is Planck's constant, υ is the frequency of X-ray beam, E_k is the kinetic energy of the electron (measured value), Φ is the energy loss constant of the individual instrument work function. Each element has a characteristic bonding energy. Measuring the intensity of the bonding energy at the specific position enables XPS to measure atomic composition of surfaces both quantitatively and qualitatively.

We expected XPS not only to reveal the fluorine content in copolymer, but more inportantly, also to reflect the efficiency of Na-K ion-exchange by determining Na/K ratio in the copolymer. Tables 5.3 to 5.5 show the atomic concentration and mass concentration of seven possible elements in the original copolymer, specimen after KF

Table 5.3: Percentage atomic concentration and mass concentration in MMA/DEA/DVB 68/30/2 blank specimen, measured with XPS.

Element	Position (eV)	Width (eV)	Atomic %	Mass %
O_{1S}	535.25	3.26	24.00	29.5
N_{1S}	399.25	1.51	2.48	2.67
C_{1S}	284.90	2.44	73.50	67.8
F_{1S}	695.95	0.05	0.00	0.00
Cl_{2P}	203.95	0.32	0.01	0.02
Na_{1S}	1076.30	0.25	0.00	0.00
K_{2P}	292.20	1.20	0.00	0.00

Table 5.4: Percentage atomic concentration and mass concentration in KF loaded MMA/DEA/DVB 68/30/2 specimen, measured with XPS.

Element	Position (eV)	Width (eV)	Atomic %	Mass %
O_{1S}	536.09	3.13	22.71	27.5
N_{1S}	399.24	2.30	2.90	3.08
C_{1S}	284.99	2.34	72.26	65.8
F_{1S}	695.54	1.36	0.55	0.79
Cl_{2P}	202.29	0.57	0.09	0.23
Na_{1S}	1076.14	0.57	1.49	2.60
K_{2P}	299.69	-0.02	0.00	0.00

Table 5.5: Percentage atomic concentration and mass concentration in Na-K ion-exchange treated MMA/DEA/DVB 68/30/2 specimen, measured with XPS.

Element	Position (eV)	Width (eV)	Atomic %	Mass %
O_{1S}	536.85	3.08	19.86	23.4
N_{1S}	399.55	1.55	2.26	2.33
C_{1S}	285.25	2.41	71.09	62.8
F_{1S}	693.25	2.76	2.06	2.88
Cl_{2P}	194.45	3.11	0.69	1.8
Na_{1S}	1076.75	0.74	4.03	6.82
K_{2P}	299.05	0.03	0.00	0.00

loading, and specimen after Na-K ion-exchange treatment, respectively. The data show marked increases in fluorine and sodium concentration after Na-K ion-exchange treatment, which clearly indicates the precipitation of the NaF in the copolymer during the Na-K ion-exchange treatment. However, no potassium was detected with XPS even in the KF loaded specimen. Also for both the KF loaded specimen and the Na-K ion-exchange treated specimen, the ratio of the sum of cation atomic concentration (Na and K) to the sum of anion atomic concentration (F and Cl) did not match the theoretical 1:1 ratio. The inadequate quantitative resolution of the XPS in this analysis could be attributed to the low sensitivity for detecting potassium, since the K_{2P} peak could be partially shaded by C_{1S} peak. Since XPS analysis did not provide us adequate quantitative precision to monitor the Na-K ion exchange process, this technique was not

further used in this project. The atomic absorbtion analysis (AA) could be the better technique for quantifying the Na-K ion-exchange efficiency.

5.2.2. Ashing

The ashing technique is simple and straight forward: after burning in air the organic portion of the specimen becomes gas, and only the inorganic component remains in the crucible. As discussed in section 5.1.3.2, we used this technique to determine the KF loading in the copolymer specimen. Low capital cost, easy operation, and quick results are the major advantages of this technique. However, the accuracy and precision of the results highly depend on the accuracy of the balance (especially when the amount of the specimen is limited), heat-stability of the inorganic component, heating history, and the practice of the operator. The data of KF loading level presented in Table 5.2 were obtained from the same batch by ashing treatment. Therefore, the results obtained are comparable because the specimens experienced the same heating/cooling history. Although the subsequent ashing experiments revealed a similar trend as indicated in Table 5.2, the absolute value varied from batch to batch. In this project, the NaF was loaded in copolymer by Na-K ion-exchange. The copolymer may contain both NaF and KF due to an incomplete ion-exchange process. The ashing technique can only provide the result of the total weight of fluoride salt loaded in the copolymer as indicated in equation 4.1. Since the molar mass of potassium is almost twice as much as that of sodium, direct interpretation of ashing results as fluorine loading levels may be misleading, unless we know the exact Na/K ratio in the copolymer.

5.2.3. Fluoride Selective Electrode

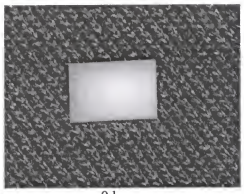
Using the fluoride selective electrode to measure the fluoride loading level was another alternative. Low capital cost and high sensitivity (< 0.1 ppm [F^-]) made this technique one of the most widely used methods for fluoride measurement The drawback of this technique is that it is time consuming. Each time of use, the fluoride electrode must be calibrated in a series of standard solutions containing fluorine ions at known concentrations. Also, this method is susceptible to interference from certain ions, such as hydroxyl group (OH^-). Therefore, before each measurement, the solution must be balanced with TISAB. To measure the fluoride loading level with the fluoride electrode, the loaded fluoride in the copolymer was allowed to release in a proper medium. By measuring the electric potential across a crystal membrane on the tip of the electrode, this method indirectly measured the fluorine ion concentration [F^-] in the solution. Therefore, the type of cation in the fluoride salt would not interfere with the results. The fluoride loaded in the copolymer was computed as in equation 4.2. Absolute fluorine loading level may not be obtained by this method, since a portion of loaded fluoride may be entrapped inside the copolymer, which was not available for release. However, since only those fluorine ions which could be released from the copolymer would function as therapeutic agent against caries, this measurement provides a meaningful fluoride measurement within the scope of the current study. In the following experiments in this study, all the fluoride quantification were measured with fluoride selective electrode.

5.3. Fluoride Release from the pH-sensitive Copolymer

There are two specific goals of these tests: 1) to confirm if the pH-sensitive swelling behavior of MMA/DEA/DVB 68/30/2 copolymer can be used as a pH-sensitive gate to accomplish on-off release of fluorine ion in response to environmental pH changes; 2) to determine if loading of a strong electrolyte in the pH-sensitive copolymer imposed a significant influence on the swelling behavior of the copolymer matrix. Two sets of test were performed accordingly.

5.3.1. pH-sensitive Release From 0.25 %wt Fluorine Loaded MMA/DEA/DVB 68/30/2 Copolymer in Lactic Buffers.

To test the fluorine ion permeability of the copolymer matrix, the possible influence of a loaded strong electrolyte on the swelling behavior of the copolymer matrix was minimized by using low fluorine loaded MMA/DEA/DVB 68/30/2 specimens which were denoted as Group A in section 4.6.1. The specimens in Group A were loaded with KF only. The fluorine loading level in these specimens was about 0.25%w/w as determined with a fluoride selective electrode. The fluoride study was performed as described in section 4.6.2. With the fluoride release, a marked swelling of the specimen was also observed at low pH. The edges of the specimen became transparent and the size of white fluoride loaded core reduced with time. Figure 5.5 shows the sequence of a typical visual observation of the specimen during fluoride release study. The size of the fluoride loaded core was reduced much faster at low pH. By measuring fluorine concentration in the receiving media with a fluoride electrode, a pH-sensitive release profile of fluorine ion from the MMA/DEA/DVB 68/30/2 copolymer in 0.1 M lactic



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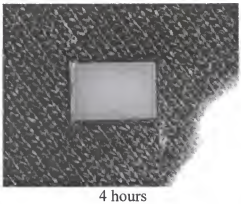
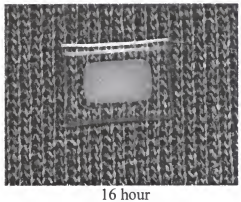
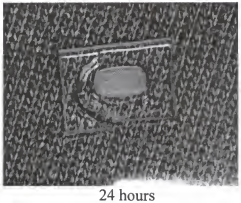


4 hours



16 hour



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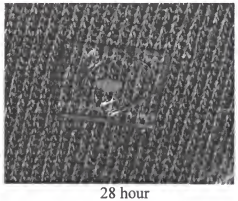
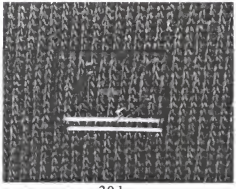


28 hour



30 hours

Figure 5.5: Sequence of a typical visual observation of the specimen during fluoride release from MMA/DEA/DVB 68/30/2 copolymer in 0.1 M lactic buffers (pH=4.0).

buffers was obtained accordingly (Figure 5.6). Generally, the release of the fluoride ceased after the disappearance of the fluoride loaded core in the copolymer. These results indicate that release of the fluoride corresponds to the swelling behavior of the copolymer matrix. The copolymer in a collapsed state can be used as a diffusion barrier for fluorine ions. Also, Figure 5.6 indicates that the release on-set pH from the MMA/DEA/DVB 68/30/2 copolymer was close to 5.5, the critical pH for dissolution of dentin.

5.3.2. pH-sensitive Release From 4.0 %wt Fluorine Loaded MMA/DEA/DVB 68/30/2 Copolymer in Lactic Buffers.

To explore the possible influence of loading of strong electrolyte in the pH-sensitive copolymer on the swelling behavior of the copolymer matrix, the fluoride release study was repeated by using high fluorine loaded MMA/DEA/DVB 68/30/2 specimens which was denoted as Group B in section 4.6.1. The fluorine loading level in these specimens was about 4.0 %wt. Visual observation was similar to the observation reported in previous section, except the disintegration of the specimens was observed for these high fluorine loaded copolymers. The swelling rate of the specimens was much faster. We believe that the high loading of a strong electrolyte (like NaF, KF) alters the swelling behavior of the pH-sensitive copolymer significantly. As shown in Figure 5.7, after the protonation of the tertiary amine and the diffusion of water molecules, the NaF started hydration and ionization. Dissolution of one micro-crystal of the NaF generated numerous sodium ions and fluorine ions, which caused a rapid increase in osmotic pressure inside the copolymer network. As discussed in Chapter 2, the increase in osmotic pressure further led to the rapid swelling of the specimen. For the high fluoride loading specimen, the osmotic force may be higher than the tensile strength of the

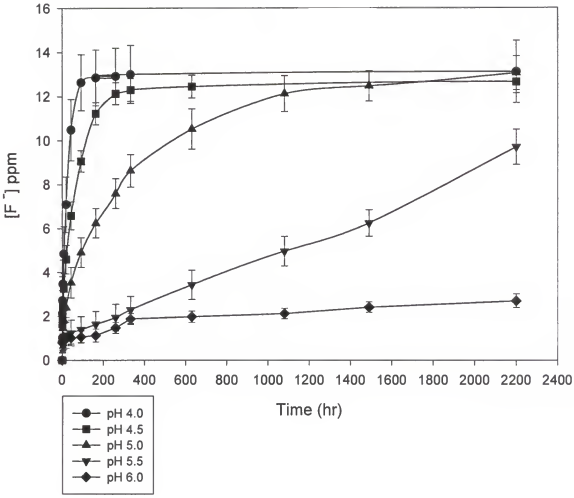


Figure 5.6: Fluoride release profiles from MMA/DEA/DVB 68/30/2 copolymer (loaded with 0.25%wt fluorine ions) in 0.1 M lactic buffer (I = 0.1 M).

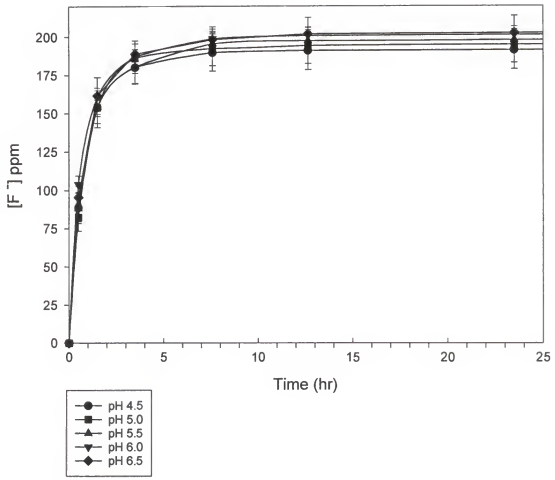


Figure 5.7: Fluoride release profiles from MMA/DEA/DVB 68/30/2 copolymer (loaded with 4.0 %wt fluorine ions) in 0.1 M lactic buffer (I = 0.1 M).

copolymer network at the swelling front, which results in the disintegration of the specimen. Accordingly, a rapid fluoride release was observed in 0.1 M lactic buffers (Figure 5.8). At all pH's investigated, the white fluoride loaded core vanished within 2 hours. The release profiles resemble each other at all pH's studied. The pH-sensitivity of fluorine release vanished at high fluorine loading level. The rapid swelling of the specimen may also be attribute to the over loading of the fluoride. As described in section 4.6.1, during the Na-K ion-exchange, the NaF was forced to precipitate in the opening micro-channels inside the copolymer. After the ion-exchange treatment, the copolymer could not regain its fully collapsed state due to the presence of NaF micro-crystals. Some of these micro-channels were still inter-connected. Therefore, as the swelling process was initiated, the water molecules could diffuse rapidly into the copolymer network through these inter-connected micro-channels.

5.4. Fluoride Release from the Bonding Adhesive Layer

To test the effect of the BisGMA/HEMA adhesive layer on the fluoride release rate, fluoride loaded pH-sensitive microspheres were used in this study. The experimental procedure was described in section 4.7. Fluorine loading level in the microspheres were determined with the fluoride electrode. The fluorine loading level in the microspheres was 0.77 ± 0.02 %wt by direct KF loading, while the fluorine loading level was 2.91 ± 0.18 %wt by the K-Na ion-exchange loading technique.

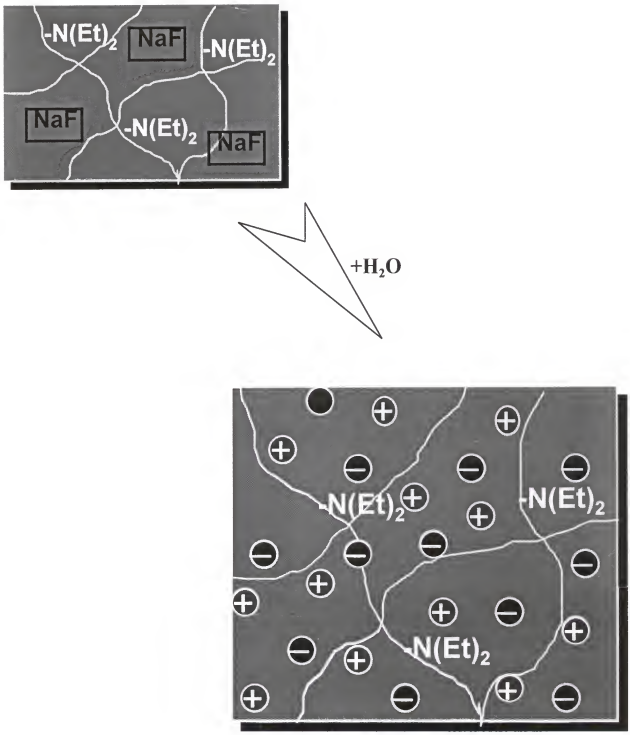


Figure 5.8: Schematical presentation of influence of loaded fluoride on the swelling of the pH-sensitive copolymer matrix.

5.4.1. Effect of BisGMA/HEMA Ratio in Adhesive on the Fluorine Permeability

To test the effect of BisGMA/HEMA ratio in the adhesive on the fluorine permeability, the pH-sensitive microspheres with 2.91%wt fluorine loading were embedded in a series of resin mixtures as listed in Table 4.4. The specimens were prepared as described in section 4.7.3. These specimens were also subjected to microsphere/adhesive interface observation. The microsphere-to-resin mixture ratio (wt/wt) was maintained at 1:2 for all the specimens. After curing with a light in a PTFE split mould, the fluoride release behavior was monitored in 0.1 M lactic buffer at pH 5.0. Figure 5.9 shows the fluorine release behavior as a function of resin component. Figure 5.10 shows the time of half release ($t_{1/2}$), the time when half of the loaded fluoride was released, as a function of BisGMA content in resin mixture. The fluoride release rate reduced rapidly with the increase in BisGMA content in the resin mixture. The result fits our initial expectation as proposed in Chapter 3. As BisGMA content increased in the resin mixture, both hydrophobicity and crosslink density increased, which resulted in less water uptake and smaller micro-channels for diffusion of the loaded fluorine ions. Theoretically, the fluorine release could be further reduced by addition of BisGMA to the resin mixture. However, further increase in the BisGMA content would cause application difficulties due to the excessive viscosity of the resin mixture. Further reduction of fluorine release rate could be achieved by addition of a more hydorphobic, less viscous crosslink agent, such as DVB, or ethylene dimethacrylate (EDMA).

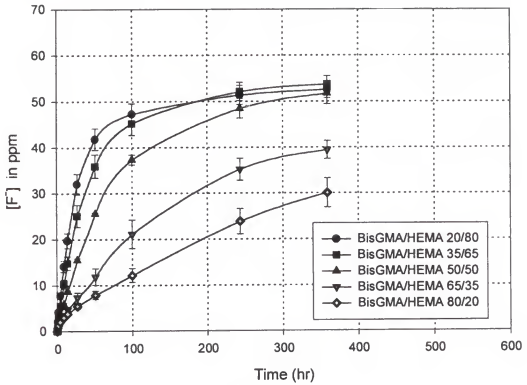


Figure 5.9: Effect of BisGMA/HEMA ratio in the adhesive on the rate of fluoride release in 10 ml 0.1 M lactic buffer at pH 5.0 (normalized to 50 mg specimen). The adhesives contain 33 wt% of fluoride loaded microspheres. Fluorine loading level in the microsphere is 2.91 wt%.

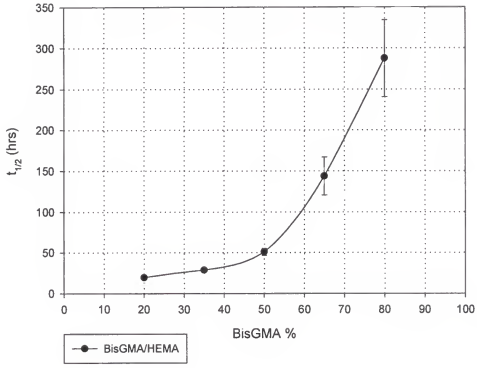


Figure 5.10: Effect of BisGMA content in BisGMA/HEMA resin mixture on the time of half release ($t_{1/2}$) for fluoride release in 0.1 M lactic buffer at pH 5.0.

5.4.2. pH-sensitive Release from Fluoride Loaded pH-sensitive Microspheres

To test the pH-sensitive fluorine release from the fluoride loaded microspheres, the pH-sensitive microspheres with both 2.91%wt and 0.77%wt fluorine loading were embedded in BisGMA/HEMA 20/80 resin mixture individually as described in section 4.7.3. To reduce of the length of the investigation, we selected a high permeable resin mixture with a low BisGMA content. Then the pH-sensitive fluorine release from these two sets of specimens was monitored in 0.1 M lactic buffer from pH 4.0 to 6.0 (in 0.5 pH increment) as described in section 4.6.2. Five replicates were made under each experimental condition. Figure 5.11 and 5.12 show the pH-sensitive fluoride release from the specimens containing 0.77%wt and 2.91%wt fluorine loaded microspheres. We observed rapid fluoride release at all pH's investigated, although the fluoride release rate was slightly lower in more basic media. Specimens in both groups failed to retain fluoride without release at pH 6.0, although no swelling was expected for unloaded MMA/DEA/DVB copolymer at that pH. The rapid fluoride release and the diminished pH-sensitivity (specially for specimens containing microspheres with high fluorine loading) could be attributed to the additional contribution of increase in osmotic pressure by dissolution of loaded fluoride as discussed before. In addition, the interaction between the BisGMA/HEMA resin mixture and microspheres may play a role in these results. The resin mixture used contained 80%wt low viscosity HEMA which is highly compatible with the MMA based microspheres. Since the size of microspheres used in the study was about 100 microns, it is highly possible that the HEMA rich resin mixture penetrated the microspheres during the mixing. At the time of curing, the microspheres

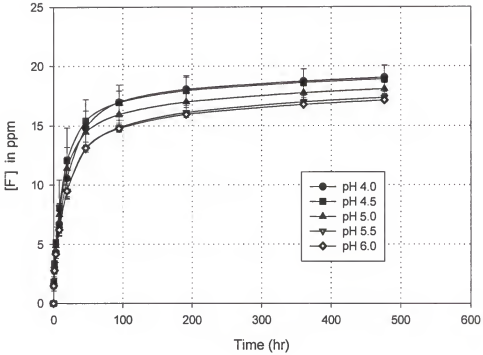


Figure 5.11: pH-sensitive fluoride release from BisGMA/HEMA 20/80 containing 33 wt% fluoride loaded microspheres in 10 ml 0.1 M lactic buffer (normalized to 50 mg specimen). Fluorine loading level in the microsphere is 0.77 wt%.

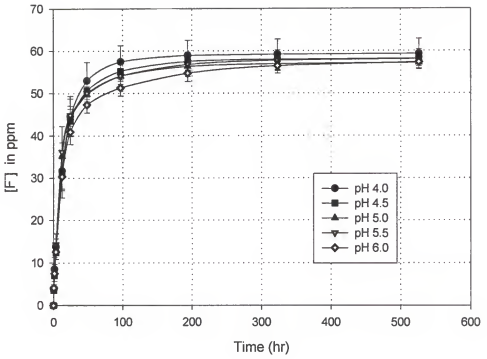


Figure 5.12: pH-sensitive fluoride release from BisGMA/HEMA 20/80 containing 33 wt% fluoride loaded microspheres in 10 ml 0.1 M lactic buffer (normalized to 50 mg specimen). Fluorine loading level in the microsphere is 2.91 wt%..

had reached their swollen state already. After curing, the HEMA rich resin mixture formed a hydrophilic inter-penetrating network through the microspheres, which provided micro-channels for rapid fluoride release, regardless pH of the environment. The resin mixture rich in BisGMA was more viscous and hydrophobic. Therefore, the microspheres remain in the collapsed state after the mixing.

5.4.3. Interface of Microspheres and Adhesive Resin Mixture

The microsphere/adhesive interface was observed with scanning electron microscope (SEM) as described in section 4.7.5. As prepared in 5.4.1, the specimens with high fluorine loading were used in this study. The purpose of this study was to observe compatibility between the microspheres and adhesive resin mixture, since bonding strength on the microsphere/adhesive interface is crucial for the overall mechanical propriety of the controlled release device.

Observing the microsphere/adhesive interface exposed by bending the specimen to fracture, we did not find any sign of debonding between microsphere and resin layer on the fracture surface of the specimens. Figure 5.13 and 5.14 show the fracture surface of specimens containing 50%wt and 80%wt BisGMA in resin mixture, respectively. The fracture went through the microspheres in all specimens tested. This observation suggested the existance of sufficient bonding along the interface of the microsphere and the adhesive layer.

Observing the microsphere/adhesive interface exposed by polishing with a series of sand paper, we found a well defined fusion zone (approximately 10 microns thick) between the microsphere and adhesive resin. Figure 5.15 and 5.16 show the polished

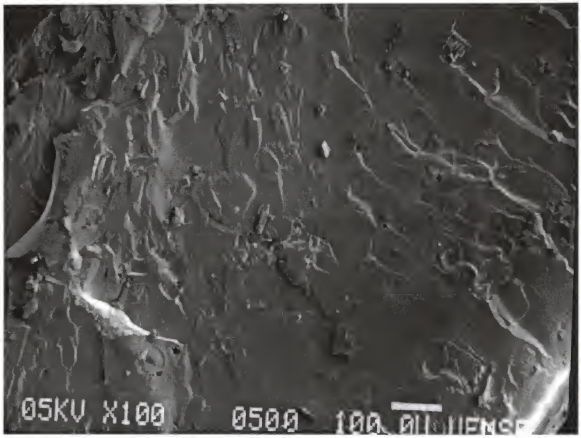


Figure 5.13: SEM observation on the fracture surface of BisGMA/HEMA 50/50 specimens embedded with fluorine loaded microspheres.

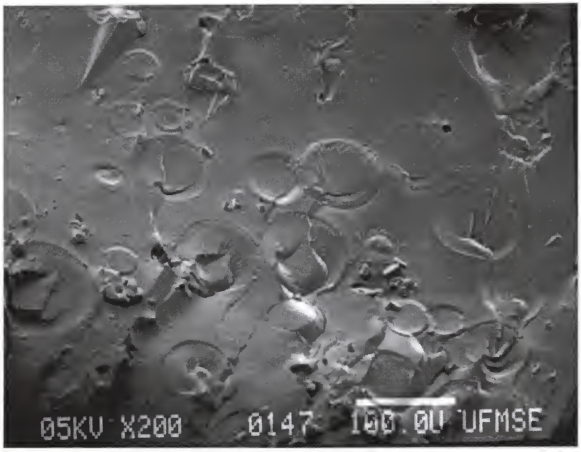


Figure 5.14: SEM observation on the fracture surface of BisGMA/HEMA 80/20 specimens embedded with fluorine loaded microspheres.

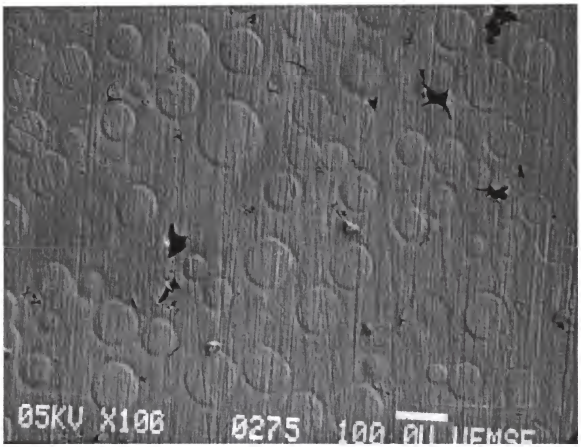


Figure 5.15: SEM observation on the polished surface of BisGMA/HEMA 20/80 specimens embedded with fluorine loaded microspheres.

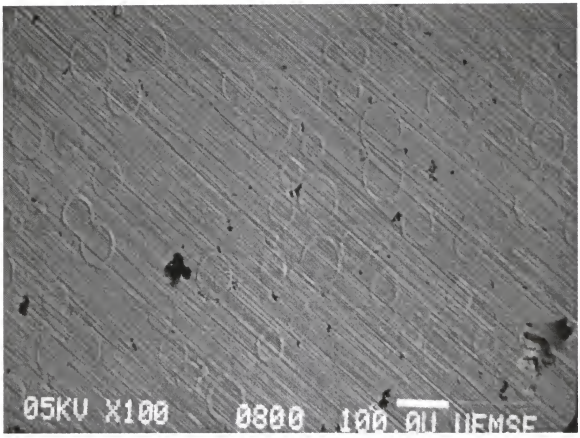


Figure 5.16: SEM observation on the polished surface of BisGMA/HEMA 80/20 specimens embedded with fluorine loaded microspheres.

surface of specimens containing 20%wt and 80%wt BisGMA in resin mixture, respectively. By close examination of the fusion zone around the microspheres, we found that the fusion zone raised from the surface for the specimens with high BisGMA content, while the fusion zone indented from the surrounding resins. This fusion zone may be caused by penetration of the resin mixture into the microspheres during the mixing. The diffusion of the resin mixture with high BisGMA content would markedly raise the crosslink density of the fusion zone of the microspheres. Therefore, a raised ring formed due to the high resistance to abrasion. In contrast, the indented ring observed in specimens with high HEMA content may be the result of a softening effect by penetration of the hydrophilic monomer into the microspheres. This result suggests that the resin with a high BisGMA content may provide stronger interfacial bonding for fluoride loaded microspheres.

5.5. Shear Bonding Strength between Dentin and Restoration

As proposed in Chapter 3, the fluoride releasing adhesive would be used in between dentin and restorative materials. Therefore, the bond quality of the resin mixture is crucial. The shear bond strength was tested as described in section 4.8. 3M ScotchBond MP was used as a control. The resin mixtures were prepared as listed in Table 4.4. The bond strength of the resin mixture with BisGMA/HEMA 80/20 was excluded from the test, due to its poor handling characteristics (excessive viscosity). The bond strength of the resin mixtures containing 33% fluoride loaded microspheres was

also tested with the same method. Five replicates were tested under each experimental condition. Figure 5.17 shows the results of the shear bond strength test.

Contrary to the effect of resin composition on the fluorine ion permeability discussed previously, the BisGMA content in the resin mixture affected the shear bond strength modestly. The results show that the shear bond strength of the resin mixture increased slightly with increasing BisGMA content up to 50%wt in the resin mixture. Further increasing the BisGMA content in the resin led to a reduced bond strength and poor reproducibility. The highest shear bond strength produced by the BisGMA/HEMA 50/50 resin was close to the value of the control group. Since BisGMA is a crosslink agent, the increasing bond strength could be attributed to the raised crosslink density in the resin. The poor handling characteristics of BisGMA/HEMA 65/35 may cause a reduction in bond strength and poor reproducibility.

After being mixed with the microspheres, the shear bond strength reduced markedly for all the resin mixture tested. The influence of resin composition on bond strength diminished further. However, as observed for resin mixtures without microspheres, reproducibility of the test reduced with increasing BisGMA content in the resin. This reduction in bond strength may be attributed to the nonuniform distribution of the microspheres in the resin mixtures. Because the density of the microspheres was higher than that of the resin mixture, during the curing, a higher distribution of microspheres was found in the region close to the dentin surface. The uneven distribution of the microspheres may cause stress concentration at that specific region, which further led to the reduction of bond strength. Furthermore, the reduction of the bond strength

may also be attributed to reduction of the effective bonding surface on the dentin due to high concentrations of the microspheres in the region close to the dentine surface.

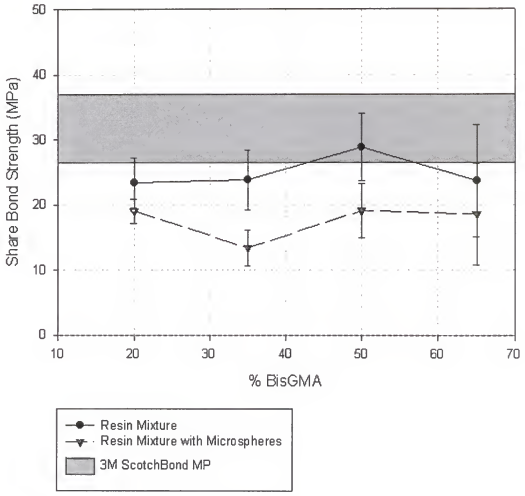


Figure 5.17: Shear bond strength of BisGMA/HEMA resin mixture on human dentine.

CHAPTER 6
CONCLUSIONS AND FUTURE WORKS

6.1. Conclusions

This study investigated two novel fluoride loading techniques based on the H-Na ion-exchange and K-Na ion-exchange processes. The K-Na ion exchange loading technique proved to be an efficient method for fluoride loading in the MMA/DEA/DVB copolymer matrix. The optimum KF loading medium was methanol/acetone in a 55/45 vol% mixture and saturated with KF. The maximum loading level achieved with this method was4.8 wt% of fluorine. The loading level could be controlled by either changing the KF concentration in loading media, or by changing of the duration of the ion-exchange treatment. The H-Na ion-exchange loading technique proved to be inefficient for the particular copolymer used in this study. Three analysis methods were compared for determination of fluoride loading levels: XPS and ashing technique proved to be efficient for a qualitative fluoride loading determination, while the fluoride electrode provided the most reliable quantitative analysis method for detecting fluorine ions.

It was determined that the MMA/DEA/DVB 68/30/2 coplymer could be used as a pH-sensitive on-off gate for controlled release of fluorine ions. pH sensitive fluorine release from the copolymer was observed with low fluorine loading. At a high fluorine loading level, the pH-sensitivity of the copolymer diminished due to the increased osmotic pressure of loading high levels of strong electrolytes. The fluorine release on-set

pH in 0.1 M lactic buffers was 5.5, which is close to the critical dissolution pH of the dentine.

BisGMA/HEMA resin mixture proved to be an effective diffusion controller for regulating the fluorine release rate. Fluorine release rate reduced markedly with increasing the BisGMA content in the resin mixture. Unfortunately, no pH-sensitive release of fluorine was observed from the specimens prepared by embedding fluoride loaded pH-sensitive microspheres in the BisGMA/HEMA 20/80 resin mixture.

Bonding between microspheres and the BisGMA/HEMA resin mixture was observed with SEM. The interface between the BisGMA/HEMA resin mixtures and the pH-sensitive microspheres was exposed with two methods. The bonding between the resin mixtures and the microspheres proved to be adequate for all the resin mixtures tested (with the BisGMA content from 20-80 %wt).

Shear bonding strength of the BisGMA/HEMA resin mixture between a sound dentine of an extracted human tooth and a composite restorative material was tested. The shear bond strength increased modestly as the BisGMA content in the resin mixture increased. The highest bonding strength (28.8 ± 5.2 MPa) was observed for the resin with 50% BisGMA Further increasing the BisGMA content in the resin led to a lower bond strength and poor reproducibility, due to excessive viscosity.

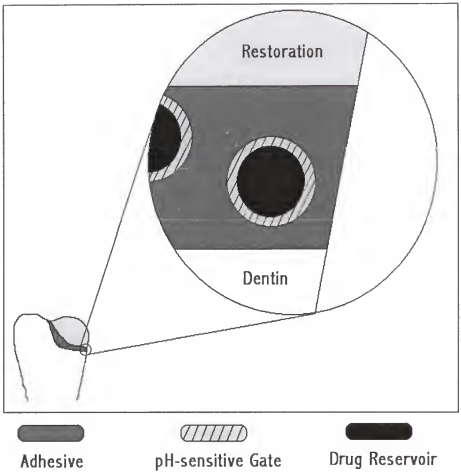
The BisGMA/HEMA resin mixtures containing 33 %wt of the fluoride loaded copolymer microspheres exhibited a marked decrease in shear bonding strength. The decrease of the shear bonding strength could be attributed to the stress concentration as well as a decrease in effective bonding area, due to the uneven distribution of the

microspheres in the resin matrix. However, except for the BisGMA/HEMA 35/65, the shear bond strengths of all other resin mixtures containing microspheres were around 19 MPa. Therefore, this fluoride releasing adhesive system would be useful to deliver fluoride ions to prevent the appearance of a white spot lesion around an orthodontic bracket, where the bond strength is less demanding.

6.2. Future Works

We were unable to fully achieve the goal proposed in chapter 1. The pH-sensitive release of fluoride from the currently designed drug delivery system was observed only with the copolymer what was loaded with 0.25 wt% fluorine. However, this study demonstrated clearly that the MMA/DEA/DVB 68/30/2 copolymer system is capable of pH sensitive release of fluoride. The fluoride on-set pH is close to 5.5. Therefore, a pH-sensitive fluoride release device could most likely be achieved by redesigning the drug delivery system.

Figure 6.1 shows a blueprint of the next generation pH-sensitive fluoride delivery system. This system should incorporate with three elements: microspheres, pH-sensitive coating, and an adhesive layer. Each of these elements would fulfil specific functions required for the fluoride delivery system. Microspheres would function solely as a drug reservoir. The pH-sensitive coating on the microspheres would serve as a pH-sensitive gate for the release of fluoride. The final fluoride release rate would be regulated by the permeability of the adhesive layer. An additional benefit of this design is that a reduced



Adhesive: BisGMA/EGDMA/HEMA 75/15/10

pH-sensitive copolymer: MMA/DEA/DVB 65/30/5

Drug Reservoir: MMA/HEMA/DVB 35/60/5

Drug: Chlorhexidine fluoride

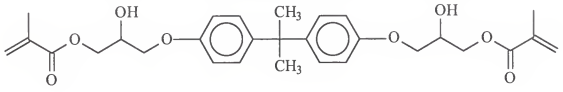
Figure 6.1: Schematical illustration of next generation of pH-sensitive fluoride release adhesive.

swelling of the adhesive would be expected, since the only the coating of the microspheres would swell in an acidic environment.

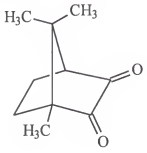
Since microspheres would function only as a drug reservoir, therefore, suggested composition for the microspheres would be MMA/HEMA/DVB 45/50/5. Copolymerization with HEMA would facilitate the loading of fluoride. Based on this study, the suggested composition for pH-sensitive coating should be MMA/DEA/DVB 65/30/5. This study also demonstrated that an increase in the hydrophobic component (BisGMA) in the adhesive layer could reduce the fluoride release rate effectively. However, further increase of BisGMA content in the adhesive would lead to an excess viscousity of the resin mixture. Therefore, other cross link agents with lower viscosity, such as ethoxylated-bisphenol-A-dimethacrylate, could be used. The adhesive could also be formulated by mixing BisGMA with other less viscous hydrophobic crosslink agents, such as ethylene glycol dimethacrylate (EGDMA). Therefore, the suggested composition for adhesive layer would be BisGMA/EGDMA/HEMA 75/15/10. Using monomers from the acrylic family could ensure the miscibility between microspheres, pH-sensitive coating, adhesive layer and other acrylic based dental resins. Finally, to enhance the therapeutic effect of released drug, chlorhexidine fluoride would be considered as the loaded fluoride, since both anions (fluorine ions) and cations (chlorhexidine) could function as anti-caries agents. In addition, due to the presence of organic cations, a high fluoride loading might be achieved with a traditional solvent loading technique. Development of a technique to coat the microspheres with a pH-sensitive membrane remains the biggest challenge for this design.

APPENDIX A
MOLECULAR STRUCTURES

2,2-bis [4(2-hydroxy-3-methacryloyloxy-propyloxy)-phenyl]propane (BisGMA):

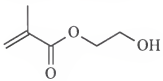


Camphorquinone:

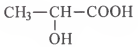


Divinylbenzene (DVB):

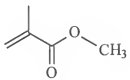
Hydroxylethyl methacrylate (HEMA):



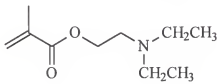
Lactic acid:



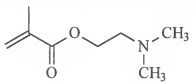
Methyl methacrylate (MMA)



N, N-diethylaminoethyl methacrylate (DEA):



N, N-dimethylaminoethyl methacrylate (DMA):



APPENDIX B
LONGEVITY OF FLUORIDE RELEASE

The most frequently asked question is how long the elevated fluoride release can be maintained from the pH-sensitive fluoride release adhesive. The longevity of fluoride release is attributable to many factors. Some of these factors are: initial fluoride loading level, fluoride release rate, size of the microcavity between the dentin and the restoration, and rate of clearance. Since most of these factors have not been established quantitatively, the following estimation is based on a series of assumptions:

1) weight percentage of fluoride loaded microspheres incorperated in the adhesive (Ms%) is 33 wt%;

2) the density of cured adhesive (ρ) is 1.2 g/cm^3;

3) fluorine loading level in microspheres (F%) is 4.0 wt%;

4) all loaded fluoride is available for release;

5) radius of cavity (r_1) is 1.5 mm;

6) thickness of adhesive (H) is 1.0 mm;

7) radius of microcavity beneath the restoration (r_2) is 1.5 mm;

8) thickness of microcavity between the adhesive and dentin (h) is 0.2 mm;

9) fluoride clearance rate in microcavity (N) is 10 times per day;

10) the fluoride concentration in microcavity (C) is maintained constantly at 5 ppm as shown in figure 1.1.

The longevity of fluoride release from the fluoride release adhesive (D) is estimated in days using the following equation:

$[(\pi r_1^2)\ H\ \rho\ Ms\%\ F\%]x10^3 = D\ [(\pi r_2^2)\ h\ C\ N]x10^{-3}$

$[(\pi \times 1.5^2) \times 1.0 \times 1.2 \times 33\% \times 4.0\%]x10^3 = D\ [(\pi \times 1.5^2) \times 0.2x\ 5 \times 10]x10^{-3}$

$D = 1854$ (days)

Therefore, constant fluoride release at 5 ppm is available for 4.3 years under these assumptions.

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